

## REMARKS

Rejection of claims 61 to 63 and 68 to 72 under 35 U.S.C. § 102(e) as being anticipated by Ebner, et al., U.S. 2003/0003545

In the June 4, 2003 Office Action, claims 61 to 63 and 68 to 72 are rejected under 35 U.S.C. § 102(e) as being anticipated by Ebner, et al., U.S. 2003/0003545 ("Ebner et al."). The Examiner maintains that Ebner et al., disclose a "polynucleotide sequence, SEQ ID NO:28, which is 99.8% identical to SEQ ID NO:4 of the present invention, and encodes a polypeptide of IL-21 (SEQ ID NO:29) having a 100% sequence identity to the amino acid sequence of SEQ ID NO:3 of the present application." See, pages 2 and 3 of Office Action dated June 4, 2003, incorporating views expressed on page 3 of Office Action dated February 25, 2003. The Examiner further asserts that claims 61 to 63 and 72 directed to a nucleic acid comprising DNA at least 95% identical to the sequence from position 50-640 of SEQ ID NO:4 or to a nucleic acid encoding amino acid residues 1-197 or 19-197 of SEQ ID NO:3 are, therefore, anticipated. Additionally, the Examiner asserted that because Ebner et al., teach a vector comprising said nucleic acid, an *E. coli*, CHO, yeast or Sf9 host cell thereof, and a process for producing the polypeptide encoded by the polynucleotide claims 68 to 71 are also anticipated.

The Examiner also maintains that Ebner et al., is prior art under §102(e) due to the existence of claims under 35 U.S.C. §119(e) in the Ebner et al., application, to certain earlier-filed provisional applications. In particular, the Examiner observes that Ebner et al., claim priority under 35 U.S.C. §119(e) to three provisional applications, No. 60/087,340, filed May 29, 1998; No. 60/099,805, filed Sept. 10, 1998; and No. 60/131,965, filed April 30, 1999.

Applicant respectfully traverses this rejection.

As an initial point, Applicant notes that the first of the three provisional applications to which claims of priority are made under 35 U.S.C. §119(e) in Ebner et al does not, in fact, disclose the nucleic acid or polypeptide sequences cited by the Examiner (i.e., SEQ. ID No. 28 and SEQ ID No. 29). The sequences labeled as SEQ ID NOS: 28 and 29 of Ebner et al., first appeared in the provisional application filed by Ebner et al., on September 10, 1998; namely, provisional application no. 60/099,805 (the '805 application ). As such, the Ebner et al., disclosure cannot be given a prior art effective date under § 102(e) for the recited sequences

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Filed: May 10, 2001

(SEQ ID NOS: 28 and 29) as of the first-claimed filing date of the '340 application (i.e., May 29, 1998).

Applicant notes that it is well-settled law that a patent (and, by implication, a patent application published pursuant to 35 U.S.C. 122(b)) shall have effect under 35 U.S.C. § 102(e) as of a particular date only to the extent that there is a sufficient disclosure under 35 U.S.C. § 112, first paragraph, for the subject matter in question. If the patent or published application claims the benefit under 35 U.S.C. § 120 (and by implication under 35 U.S.C. § 119(e)) to an earlier filed application, that patent or published application shall not be entitled to prior art effect under § 102(e) if the earlier filed application does not provide a sufficient disclosure under 35 U.S.C. § 112, first paragraph for the subject matter in question. To be given effect under § 102(e), the claims of the reference patent must be supported in the manner required by 35 U.S.C. § 112 in the priority application whose date is relied on to establish the prior art status of the patent. *See In re Wertheim*, 646 F.2d 527, 209 USPQ 554 (CCPA 1981); and MPEP 2136.03, sub-heading IV.

Applicant also directs the attention of the Examiner to the declaration submitted pursuant to 37 CFR § 1.131, executed by the inventors of the present application, and provided herewith. Applicant submits that the declaration effectively antedates the Ebner et al., reference, particularly in view of the observations provided above which establish that Ebner et al., is not entitled to a prior art date pursuant to 35 U.S.C. 102(e) of May 29, 1998 (i.e., the filing date of the '340 application).

Accordingly, Applicants submit that Ebner et al., is not prior art to the presented claims under § 102(e) and as such, cannot be relied upon to support a § 102(e) rejection of said claims. *See* MPEP § 2136.05. Applicant respectfully request the Examiner to withdraw the rejection of claims 61-63 and 68-72 under § 102(e) rejection based on Ebner et al.

Rejection of Claims 61-72 under 35 U.S.C. § 102(e) as being anticipated by Gorman, et al., US Patent No. 6,562, 578 B1

In the June 4, 2003 Office Action, claims 61 to 63 and 68 to 72 were rejected under § 102(e) as being anticipated by Gorman et al., US Patent No. 6,562, 578 B1 (the '578 patent). The Examiner maintains that Gorman et al., disclose a nucleic acid, Seq. ID NO. 22, that comprises nucleotides 50 to 640 of SEQ ID NO:4 of the present invention with 100% sequence identity and encodes a human polypeptide of SEQ ID NO:23 with 100% identity to SEQ ID



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NO:3 of the present invention. The Examiner further maintains that Gorman et al., teach a vector containing said nucleic acid, an E. coli, yeast and mammalian host cell comprising said sequence, and a method of producing the polypeptide encoded by said recited nucleotide sequence. Upon this basis the Examiner maintains that Gorman et al., anticipate claims 68 to 71 of the instant application.

Applicant respectfully traverses this rejection.

Gorman et al., was filed on January 10, 2000, and makes a claim under 35 U.S.C. 119(e) to U.S. provisional application no. 60/115,506 (the '506 application), filed on January 11, 1999. The '506 application, however, does not disclose the nucleic acid or polypeptide sequences labeled as SEQ ID NO: 22 and 23 in the '578 patent. No other sequences bearing the recited homology to the presently claimed nucleic and polypeptide sequences are disclosed in the '506 application. Accordingly, Gorman et al., is not entitled to have an prior art effect under §102(e) for the nucleic acid and polypeptide sequences labeled as SEQ ID NO: 22 and 23 in the '578 patent prior to the actual filing date of the '578 patent (i.e., January 10, 2000).

Applicant further notes that the effective filing date of the present application is prior to January 10, 2000 by operation of 35 U.S.C. §§119 and 120. As such, Gorman et al., is not prior art to the presented claims under 35 U.S.C. 102(e).

Accordingly, Applicant respectfully requests the Examiner to withdraw the rejection of claims 61 to 72 under 35 U.S.C. 102(e) based on Gorman et al.

Rejection of claims 64 to 67 under 35 U.S.C. § 102(e) as anticipated by, or in the alternative, under § 103(a) as obvious over Ebner, et al., US 2003/0003545.

In the June 4, 2003 Office Action, claims 64 to 67 are rejected under § 102(e) as anticipated by, or in the alternative, under §103(a), as obvious over Ebner et al., US 2003/0003545 (the '545 application). In the Office Action, the Examiner maintains that polynucleotide sequence of SEQ ID NO:28 disclosed in Ebner et al., is 99.8% identical to the nucleotides 50-640 or SEQ ID NO: 4 of the present invention with a single mismatch at position 385. The Examiner further maintains that the mismatch does not change the encoded amino acid at that position. The Examiner also requests Applicant to provide evidence that the prior art would neither anticipate nor render the claimed invention obvious.

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Filed: May 10, 2001

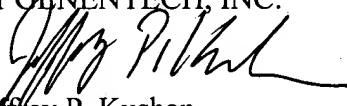
For the reasons presented above in relation to the rejection of claims 61 to 63 and 68 to 72, Applicant maintains that Ebner et al., is not prior art under 35 U.S.C. § 102(e) to the present application. As such, Applicants respectfully request the Examiner to withdraw the rejection of claims 64 to 67 under §102(e) and/or §103 based on Ebner et al.

Additional Comments

Applicants, for the convenience of the Examiner, have enclosed copies of the applications referred to in the declaration submitted pursuant to 37 CFR 1.131. Specifically, said copies are attached as exhibits to said declaration.

In view of this response, Applicant submits that the present application is in condition for allowance and should be passed to issue. If the Examiner believes that the application is not in condition for allowance or cannot be passed to issue in view of this response, Applicant respectfully requests that the Examiner contact the undersigned prior to taking any further action in this application.

Respectfully submitted,  
for GENENTECH, INC.

  
Jeffrey P. Kushan  
Registration No. 43,401

Date: 10/29, 2003



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

|   |   |
|---|---|
| In re Application of Jian Chen, <i>et al.</i>       | Group Art Unit: 1646                    |
| Serial No.: 09/854,280                              | Examiner: Jiang Dong                    |
| Filed: May 10, 2001                                 |   |
| For: IL-17 Homologous Polypeptides and Uses Thereof | Attorney Reference: P1381R1C2/22338-403 |

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NOV 06 2003  
TECH CENTER 1600/2900

**RULE 131 DECLARATION**

I, Jian Chen, Ellen Filvaroff, Audrey Goddard, Austin Gurney, Hanzhong Li and William I. Wood, hereby declare as follows:

1. I am one of the six named inventors of the claimed subject matter of the above-identified patent application.
2. The above-identified patent application claims priority to application serial no. 09/311,832 with the Patent Office on May 14, 1999, and I am a named inventor in that priority application. A copy of the priority application is attached as Exhibit A.
3. The above-identified patent application also claims priority to two provisional applications, 60/085,579 and 60/113,621, filed on May 15, 1998 and December 23, 1999, respectively.
4. All work described in the above-identified application and the priority applications was performed by me or one of the other named inventors (or on our behalf) in the United States of America.
5. I have read and reviewed US Patent Publication No. 2003/0003545 published to Ebner *et al.* on January 2, 2003 (hereinafter the "545 publication") (a copy of which is attached as Exhibit B). I understand that the '545 publication is based on application serial no. 09/320,713 filed with the Patent Office on May 27, 1999 and claims priority to three different priority provisional applications filed in the Patent Office in the time period between April 30, 1999 and May 29, 1998.
6. I have also read and reviewed the provisional applications referred to in the '545 publication; namely, No. 60/087,340, filed May 29, 1997 (attached as Exhibit C); No. 60/099,805 filed Sept. 10, 1998 (attached as Exhibit D); and No. 60/131,965 filed April 30, 1999 (attached as Exhibit E).
7. Based on my review of the earliest filed provisional application, No. 60/087,340, I conclude that this application does not disclose the nucleic acid sequence or the polypeptide sequence (i.e., SEQ ID NO:28 and 29) cited by the Examiner as a basis to

reject the claims of the above-identified application. Instead, these two sequences, which the Examiner indicates as corresponding to SEQ ID NO:3 and 4 of the above-identified application, appear only in the second provisional application, No. 60/099,805 (hereinafter the "805 application"), filed September 10, 1998, and in applications to which the '545 application claims priority filed after September 10, 1998.

8. Prior to September 10, 1998, I, one of the other named inventors or a person acting on our behalf had identified an EST with a sequence similarity to "cytotoxic T-lymphocyte-associated antigen 8" (another name for IL-17)(entered into applicant's proprietary database as DNA number DNA49665), isolated the cDNA clone of such sequence (internal reference No. IL-17C-DNA62377)(notebook No. 29129, pgs. 37-42, 46, 48, 53-54), sequenced the cDNA for the full length clone (Designated DNA No. DNA62377), designed and prepared oligonucleotide primers to enable construction of protein expression vectors for IL-17C-DNA62377 (notebook No. 29416, p. 39), prepared an expression vector to enable production of IL-17C as an fc fusion protein using baculovirus (notebook No. 30469, p. 28) and confirmed protein expression via small-scale studies using the vector (notebook No. 30469, p. 53), performed large-scale protein expression experiments and purified the expressed IL-17C protein (notebook No. 30439, pgs. 35, 49-50 and notebook No. 30518, as noted in Applicant's proprietary database). See attached Exhibit F for documents demonstrating the above-described experiments.

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I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10/9/2003  
Date

Jian Chen  
Jian Chen

\_\_\_\_\_  
Date

\_\_\_\_\_  
Ellen Filvaroff

\_\_\_\_\_  
Date

\_\_\_\_\_  
Audrey Goddard

reject the claims of the above-identified application. Instead, these two sequences, which the Examiner indicates as corresponding to SEQ ID NO:3 and 4 of the above-identified application, appear only in the second provisional application, No. 60/099,805 (hereinafter the "805 application"), filed September 10, 1998, and in applications to which the '545 application claims priority filed after September 10, 1998.

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\*\*\*\*\*

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\_\_\_\_\_  
Date

9/5/03


\_\_\_\_\_  
Date

\_\_\_\_\_  
Jian Chen

  
\_\_\_\_\_  
Ellen Filvaroff

9/17/03

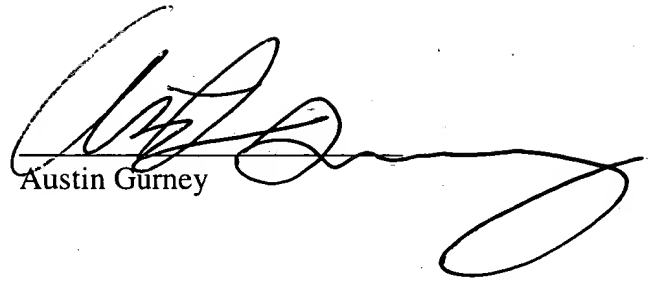
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Date

  
\_\_\_\_\_  
Audrey Goddard

8/29/07  
Date

\_\_\_\_\_  
Date

\_\_\_\_\_  
Date

  
Austin Gurney

\_\_\_\_\_  
Hanzhong Li

\_\_\_\_\_  
William I. Wood

\_\_\_\_\_  
Date

9/6/03  
Date

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Austin Gurney

Han Li  
Hanzhong Li

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William I. Wood

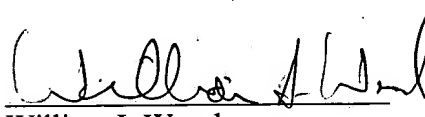
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Austin Gurney

\_\_\_\_\_  
Date

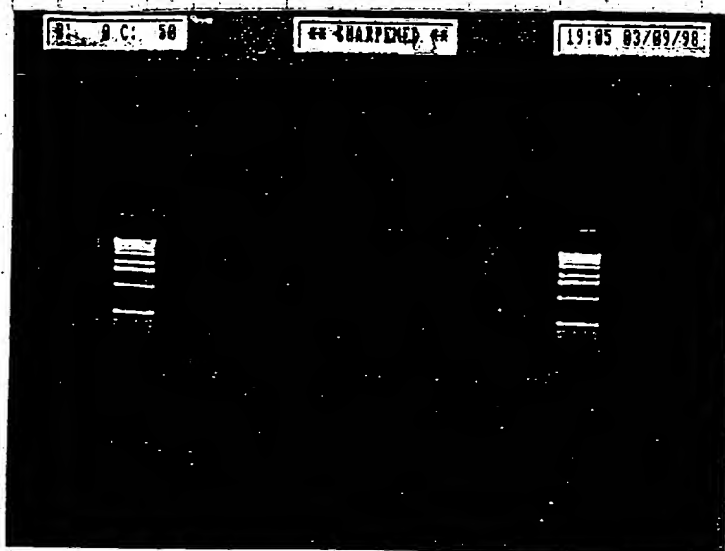
\_\_\_\_\_  
Hanzhong Li

9/19/02  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
William I. Wood



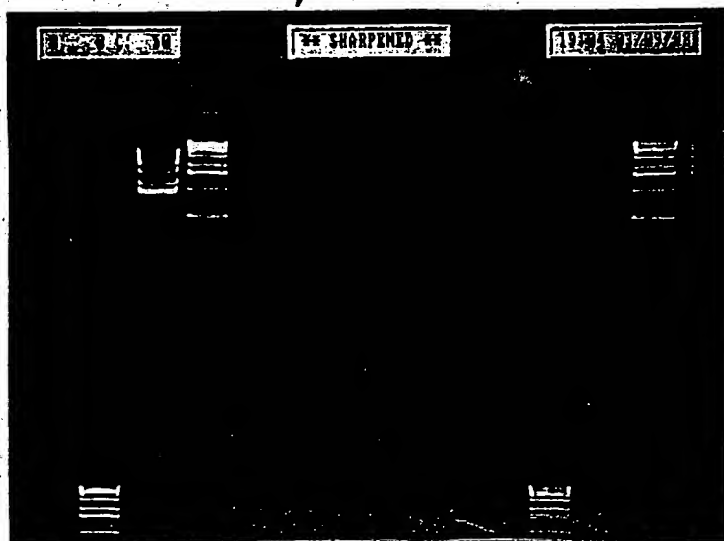
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sourcing for 49665

18  
 1> 100ng ssb  
 90 5> blentag buffer  
 18 1> dNTP  
 18 1> forward  
 18 1> reverse  
 18 1> blentag  
 720 40> ddH<sub>2</sub>O  
 50> total

49665  
 200bp



95°C 5min 1 cycle

95 1min  
 57 1min  
 72 1.5min

20 cycles

1.5% TAE gel

228 may possibly  
 have the band

thoughts try DMSO w/ amplitag and see if  
 this helps

T Page No. \_\_\_\_\_

Witnessed &amp; Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by

Buthanne Doud

Recorded by \_\_\_\_\_

Date \_\_\_\_\_

PCR sourcing w/ 5% DMSO &amp; taq

20

1x ss. lib

5x buffer

1100

3x MgCl<sub>2</sub>

100 100

2.5x DMSO

55

1x forward

220

1x reverse

220

1x dNTP

220

1x amplitas

220

to 50x ddH<sub>2</sub>O

759

95°C 5 min 1 cycle

95° 1 min

56° 1 min

72° 1 min 25 cycles

product should be around 200bp

227 228 294 possibilities

run gel and do Southern  
order

228 from yesterday

229D

26

229

94

230

99

247

135

247

153

255

154

293

227D

294

227

301

228

302

~~229D~~~~228~~

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Witnessed &amp; Understood by me,

Date

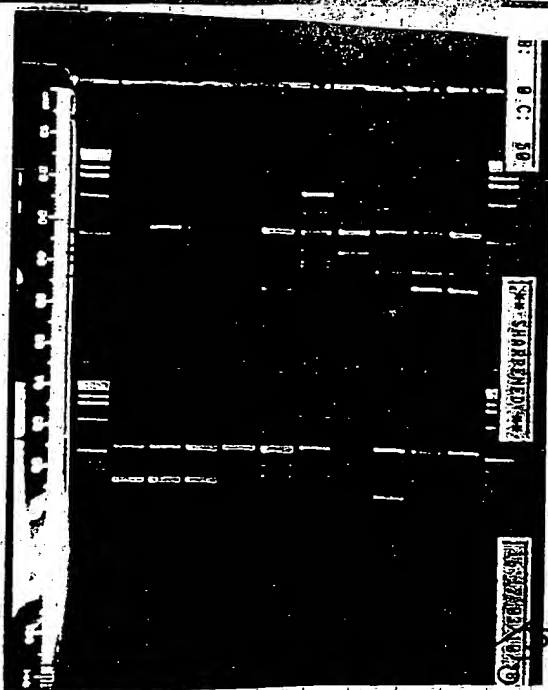
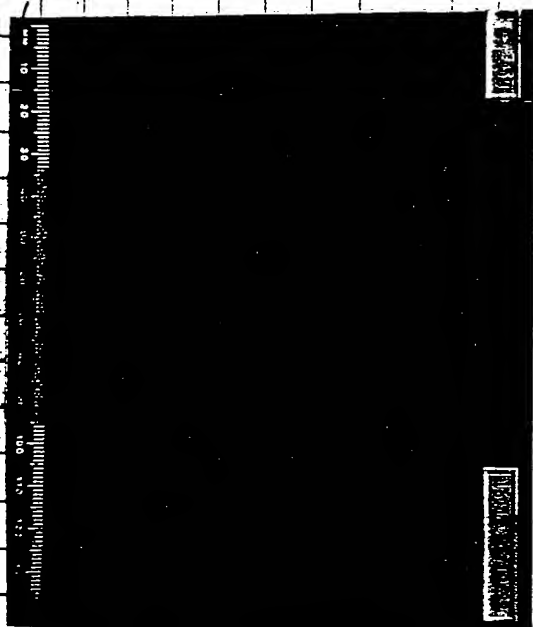
Initiated by

B. Thanne Zewel

Date

Recorded by

From Page No. \_\_\_\_\_



just comps add 500x transfection + 500x compl  
let rock like add 10 ml media

1 56436  
5 57695  
9 56350  
15 57694  
17 57834  
4 58800

let go until Friday  
when harvest cells.

Along pts Southern for 49165 cytokine  
Probably 229 or 228 has it  
If not try 254 etc

Take apart Southern. Neutralize and bake  
2-3 hrs in 80° vacuum oven

To Page No. \_\_\_\_\_

Witness d &amp; Understood by me,

Dat

Invented by

Bithanne Deneil

Date

Recorded by

From Page No. \_\_\_\_\_

PAGE: 1

USER: 5 ID: 32P MAXI COMMENTS:  
 PRESET TIME: 1.00 H#: YES SAMPLE REPEATS: 1 DATA CALD: CPM  
 PRINTER: STD SCR: NO REPLICATES: 1 COUNT BLANK: NO  
 RS232: OFF RCM: YES MULTIPLIER: 1.000000 VIAL SIZE: MAXI

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE: YES

| SAM NO | POS | TIME MIN | H#   | 32P CPM  | %ERROR | RCM  | ELAPSED TIME |
|--------|-----|----------|------|----------|--------|------|--------------|
| 1      | 1-1 | 0.51     | 49.0 | 86387.21 | 0.95   | 0.00 | 1.38         |

make probe 49665

1x 49665 pl  
 10x 8<sup>32</sup>P ATP  
 6x T4 pph buffer  
 2x T4 pph  
 41x ddH<sub>2</sub>O

mix and incubate 30 min 37°C  
 clean  
 dilute 1:10 and count

Miniprep + and do Xba Digests

|                        |        |
|------------------------|--------|
| 5x cDNA                |        |
| 2x buffer              | HNH402 |
| 1x Xba                 | 64     |
| 12x ddH <sub>2</sub> O | 32     |
|                        | 384    |

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Witnessed &amp; Underst d by m ,

Dat

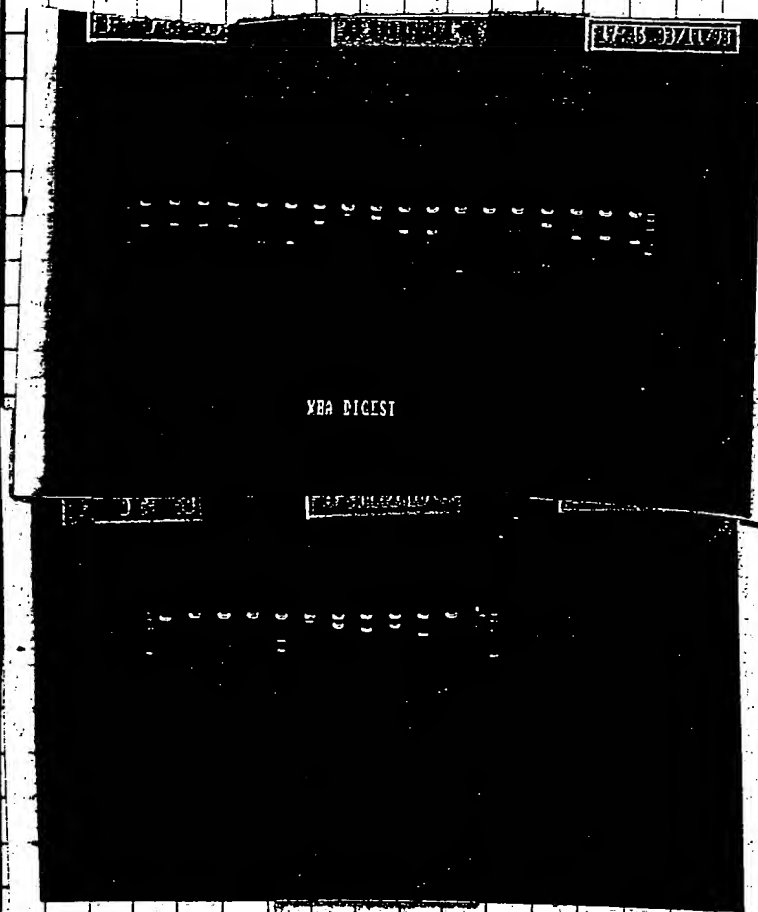
Inv nted by

B. Thanne Deuel

Dat

Recorded by

From Page N



52159

(3)

(4)

(5)

(7)

any

52721

3

(4)

(5)

52165

2 (M)

(3)

52163

(2)

(3)

any

52175

(1)

(3)

(4)

any

52035

1

(2)

(4)

(5)

any

52161

1

(2)

(7)

52772

(4)

(5)

replate peng

52765

(1)

(2)

(3)

52162

(1)

replate

52160

1

42836

2

→ ?

To Page No.

Witnessed &amp; Understood by me,

Dat

Inv nt d by

Bethanne Daniel

Rec rd d by

Dat

om Page No. \_\_\_\_\_

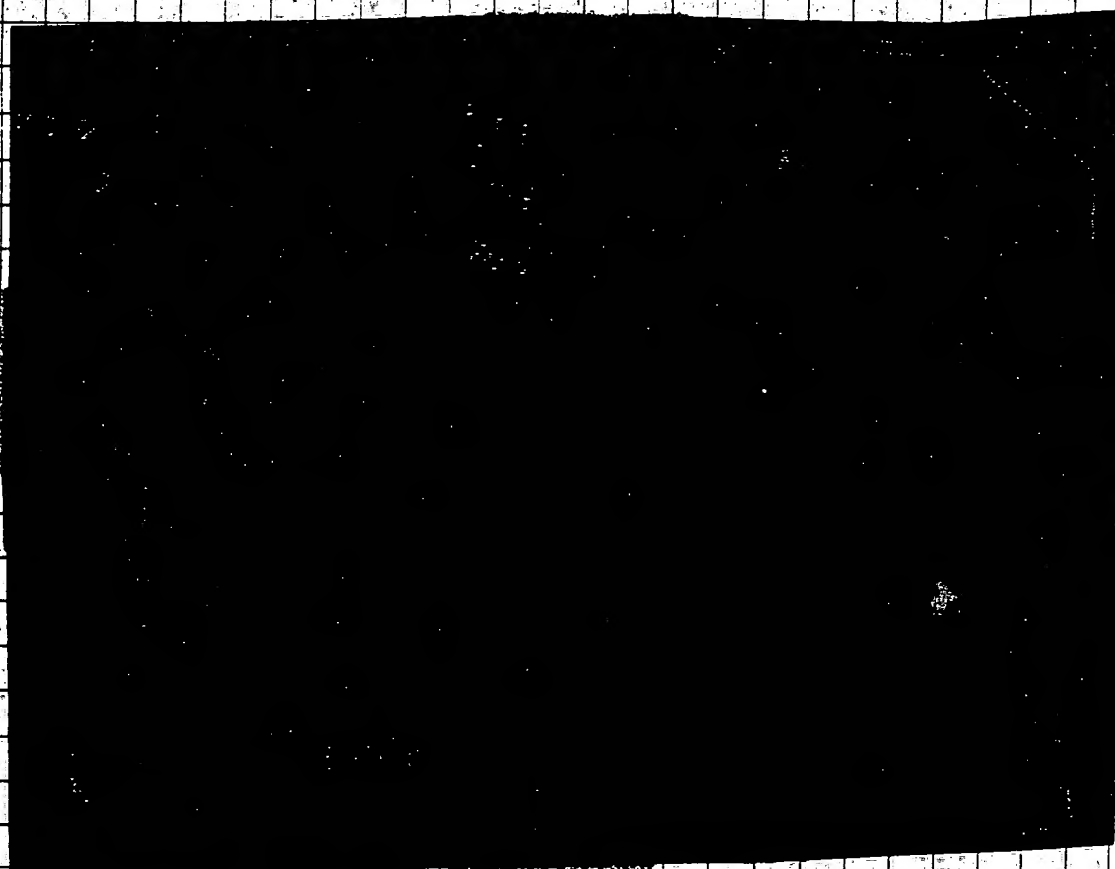
Wash Southern

2XSSC + 0.1% SDS 42° 40 min

0.5XSSC + 0.1% SDS 42° 15 min

0.1XSSC + 0.1% SDS 42° 10 min

expose to film 2 hrs develop

Source for cloning 49665  
227, 228, 294

To Page No. \_\_\_\_\_

Inspected &amp; Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invited by \_\_\_\_\_

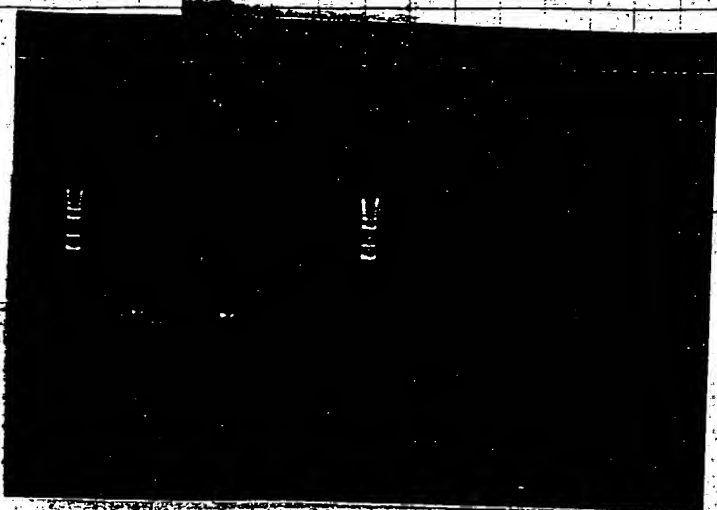
Bethanne Deuel

Recorded by \_\_\_\_\_

Date \_\_\_\_\_



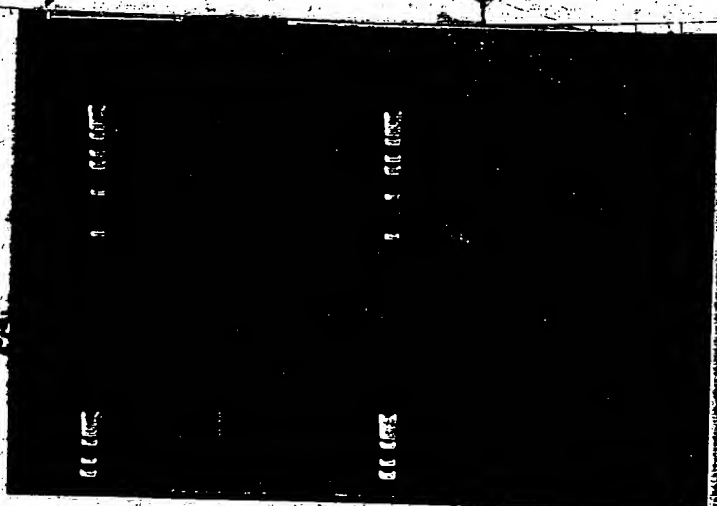
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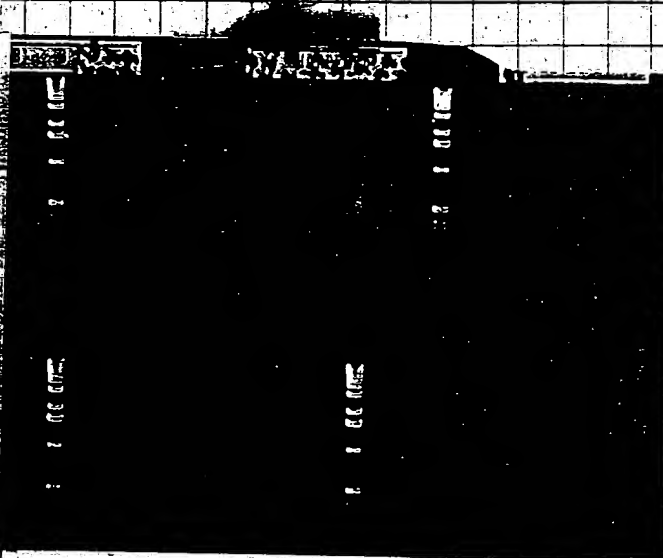
38237

1036

184? 247? 255?



38085 - 292bp 462bp



60684 2/90

Cloning

~ 49665 - wash buffers

2XSSC + 0.1% SDS 40 min

0.5XSSC + 0.1% SDS 20 min

late air dry expose to film develop

To Page No. \_\_\_\_\_

Witnessed &amp; Und rsto d by me, \_\_\_\_\_

Dat \_\_\_\_\_

Invented by

Bethanne Deuel

Rec rded by \_\_\_\_\_

Date \_\_\_\_\_

Form Page No. \_\_\_\_\_

Sourcing  
 run PCR to check size of  
 60684  
 the bottom bands  
 are 150

so use 26, 153, 254, or 247  
 for cloning

Cloning - 1 pick + for 49665  
 grow O/N  
 $39 + \frac{26}{65} =$  in 17,000 colonies

2) transform and plate rescues from yesterday

PCR for new sourcing

60748 - 160 bp 56° annealing

38085 - 8 kb 12 diff annealing temp

95° 5 min 1

95° 1 min

52-63° 1 min

72° 1 min 10 sec 25

72°C 5 min 1

T Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

Invented by

Date

Recorded by



From Page No. \_\_\_\_\_

do miniprep on 49665

Baculovirus

Set up 1st amp

Cloning

make probes for

|        |       |       |       |                               |     |
|--------|-------|-------|-------|-------------------------------|-----|
| 52781  | 31456 | 26967 | 10974 | 0.5 $\mu$ probe               | 12  |
| 601684 | 28096 |       |       | 3 $\mu$ pnc buffer            | 31  |
| 60748  | 36461 |       |       | 5 $\mu$ $\gamma$ $^{32}$ PATP | 60  |
| 38085  | 35735 |       |       | 2 $\mu$ T4 pnc                | 24  |
| 38237  | 26963 |       |       | 19.5 $\mu$ ddH <sub>2</sub> O | 234 |

clean probes in Puroluant G<sup>50</sup> micro columns  
freeze

Set up PCR for sourcing of 14 oligos in  
12 libraries

plate in vivo from yesterday

Set up PCR for reverse of 49665

|                             |  |     |
|-----------------------------|--|-----|
| 1 $\mu$ bugs                |  | 12  |
| 5 $\mu$ ligation buffer     |  | 60  |
| 1 $\mu$ forward 49665       |  | 12  |
| 1 $\mu$ reverse 49665       |  | 12  |
| 1 $\mu$ dNTP                |  | 12  |
| 1 $\mu$ ligation            |  | 12  |
| 40 $\mu$ ddH <sub>2</sub> O |  | 480 |

cycling  
cond  
for  
broth  
except  
72°C 1.5 min  
in 20 cycles

95°C 5 min 1 cycle  
95°C 1 min  
59 1 min  
72 3 min 3 cycles  
95 1 min  
57 1 min  
72 3 min 3 cycles

95°C 1 min  
55°C 1 min  
72°C 3 min  
20 cycles

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by m ,

Dat

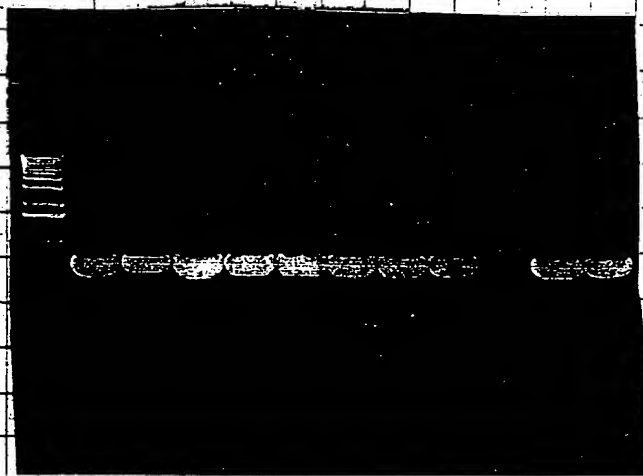
Invent d by

Bethanne Zemel

Date

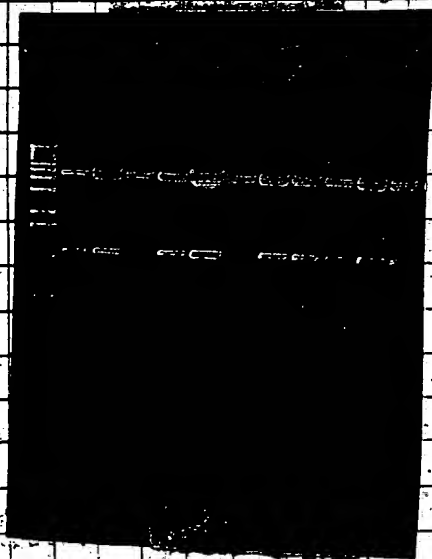
Recorded by

From Pag No. —



49665 REN

set up xba digest for 49665



|                        |     |
|------------------------|-----|
| 5x CDNA                | 12  |
| 2x React 2             | 24  |
| 1x Xba-T               | 12  |
| 12x ddH <sub>2</sub> O | 144 |

set digest 1 hr 37°C

To Pag No. —

Witnessed &amp; Understood by me,

Date

Invented by

Bethanne Zewel

Date

Recorded by

From Page No. \_\_\_\_\_

|                       |   |  |                      |
|-----------------------|---|--|----------------------|
| 59777.AH692.f OLI8260 | 1 | TTT GGG TTT GGC GCG CCC TCT CCA ATA CTG GTC AAG ACA CCA TTT                        | DNA                  |
| 45 mer                |   | reference:   |                      |
| 59777.AH694.Nsl.f     | 1 | TTG GTT ATG CAT CAG ATG ACG ATG ACA AAC TCT CCA ATA CTG GTC<br>AAG ACA CCA TTT     | DNA                  |
| 57 mer                |   | reference:   |                      |
| 59777.AH693.r OLI8262 | 1 | GTC ACC TTC GCC TAA TGT TT   | 5' Phosphorylate DNA |
| 20 mer                |   | reference:   |                      |
| 59777.AH695.Not.r     | 1 | TTT GGG TTT GCG GCC GCT TAG TCA CCT TCG CCT AAT GTT T                              | DNA                  |
| 40 mer                |   | reference:   |                      |
| 62377.AH696.f OLI8264 | 1 | AAA CCC AAA GCG GCC GCT AAA CCA CCA TGA CGC TCC TCC CC                             | DNA                  |
| 41 mer                |   | reference:   |                      |
| 62377.AH698.Nsl.f     | 1 | AAA CCC AAA ATG CAT CAG ATG ACG ATG ACA AAC ACC ATG ACC<br>CCT CCC                 | DNA                  |
| 48 mer                |   | reference:   |                      |
| 62377.AH697.r OLI8266 | 1 | CAC TGA ACG GGG CAG CAC  | 5' Phosphorylate DNA |
| 18 mer                |   | reference:   |                      |
| 62377.AH699.Not.r     | 1 | AAA CCC AAA GCG GCC GCT TAC ACT GAA CGG GGC AGC AC                                 | DNA                  |
| 38 mer                |   | reference:   |                      |
| 59814.AH700.f OLI8268 | 1 | AAA CCC AAA GCG GCC GCT AAA CCA CCA TGA GAG TGT CAG GTG<br>TGC TTC GCC             | DNA                  |
| 51 mer                |   | reference:   |                      |
| 59814.AH702.Nsl.f     | 1 | AAA CCC AAA ATG CAT CAG ATG ACG ATG ACA AAA TAG TCA CGA<br>CAT GGA TGT TTA TTC GAA | DNA                  |
| 60 mer                |   | reference:   |                      |
| 59814.AH701.r OLI8270 | 1 | AAA TGG CTT CGG GGG CAT GCA  | 5' Phosphorylate DNA |
| 21 mer                |   | reference:   |                      |
| 59814.AH703.Not.r     | 1 | AAA CCC AAA GCG GCC GCT TAA AAT GGC TTC GGG GGC ATG CA                             | DNA                  |
| 41 mer                |   | reference:   |                      |
| 60625.AH704.f OLI8272 | 1 | AAA GGG AAA GCG GCC GCT AAA CCA CCA TGG CGA ACC CCG GGC<br>TG                      | DNA                  |
| 44 mer                |   | reference:   |                      |
| 60625.AH706.Nsl.f     | 1 | AAA GGG AAA ATG CAT CAG ATG ACG ATG ACA AAC GCT GGG GCC<br>GAG                     | DNA                  |
| 45 mer                |   | reference:   |                      |
| 60625.AH705.r OLI8274 | 1 | AGC AGT GAT GGC TTC CGG A  | 5' Phosphorylate DNA |
| 19 mer                |   | reference:   |                      |
| 60625.AH707.Not.r     | 1 | AAA GGG AAA GCG GCC GCT TAA GCA GTG ATG GCT TCC GGA                                | DNA                  |
| 39 mer                |   | reference:   |                      |

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*Make maps and enter constructs in database*

### Baculovirus Construct DNA Sequencing Hard Copy

Scientist: Bethanne Deuel

Oligos: 403 and 406

| Construct DNA Number | FL DNA number | Miniprep number |
|----------------------|---------------|-----------------|
| 71298                | PH.48314.IgG  | 48314.1         |
| 71299                | PH.48314.IgG  | 48314.3         |
| 71305                | PH.49821.IgG  | 49821.9         |
| 71306                | PH.49821.IgG  | 49821.10        |
| 71291                | PH.41386.IgG  | 41386.20        |
| 71303                | PH.49624.IgG  | 49624.34        |
| 71304                | PH.49624.IgG  | 49624.35        |
| 71296                | PH.45410.IgG  | 45410.39        |
| 71297                | PH.45410.IgG  | 45410.40        |
| 71300                | PH.48333.IgG  | 48333.45        |
| 71301                | PH.45417.IgG  | 45417.49        |
| 71302                | PH.45417.IgG  | 45417.50        |
| 71294                | PH.46777.IgG  | 46777.54        |
| 71295                | PH.46777.IgG  | 46777.55        |
| 71293                | PH.44804.IgG  | 44804.64        |
| 71307                | PH.62377.IgG  | 62377.1         |
| 71308                | PH.62377.IgG  | 62377.2         |

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## Ramp Seal infections on:

|       |       |         |             |
|-------|-------|---------|-------------|
| 34434 | his   | JF      |             |
| 62306 | his   | 8       | JF          |
| 62377 | Igg   | 6/5/48  | } B.D.      |
| 62377 | Igg   | 5/27/48 |             |
| 65351 | mp#83 | B.D.    |             |
| 60274 | #67   | B.D.    | only 500mls |
| 59825 | 131   | B.D.    |             |

IL  
↓  
IL

## Amps on:

|        |      |
|--------|------|
| 58723  | 146  |
| 66309  | 59   |
| 33085  |      |
| 58737  | 1549 |
| 116451 | NLB  |
| 47394  | 4    |
| 22781  | NL3  |
| 60615  | 1530 |
| 62886  | 143  |
| 61755  | 149  |
| 64905  | 51   |
| 04885  | 135  |

Split (3) 1:8 focus -

25ml

5ml / plate

- plates &gt; 75% confluent -

wash plate 1 w/ 10ml, aspirate  
then add 5ml complete

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harvest 2nd Amps from pg 35

gels on large scales:

15% Reduced

MW  
 65405. M2. his 35 28,465  
 30868. his 39,284  
 34434. his 41,886  
 62306. his 27,163

15% Not Reduced

MW  
 65405. M2. 35  
 30868. his  
 34434. his  
 62306. his

av.  
 a.k.  
 Aggregated

12% Reduced

MW  
 1L17 6/5 62377. IgG 21,264  
 1L17 5/ " "  
 59825. IgG 30,570  
 60274 8/28 IgG 18,153  
 60274 8/31 IgG 16,454  
 62810. IgG 16,454  
 64903. IgG 10,110  
 49821. IgG 13,710  
 59847. IgG 40.454 a.k.  
 65351. IgG 11,419

12% Not Reduced

1L17 6/5 62377. IgG } expression  
 1L17 5/ " } questionable  
 59825 O.K.  
 60274 Aggregated  
 60274  
 62810 Aggregated  
 64903 O.K.  
 49821 - Aggregated  
 59847 - Aggregated O.K.  
 65351 - definitely aggregated

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Date

Inv nted by

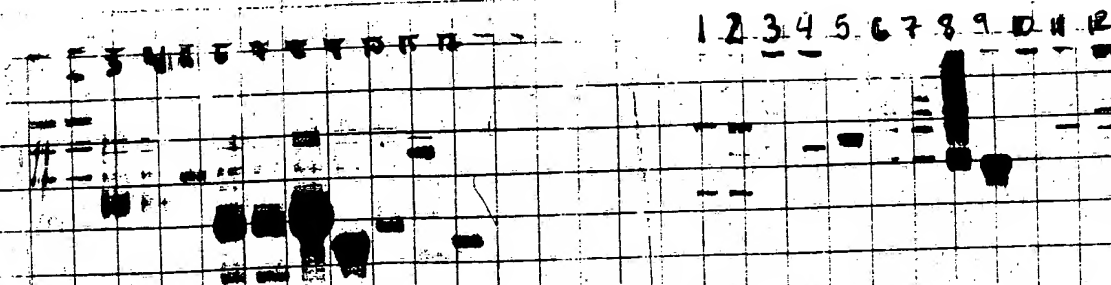
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gels



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5 **IL-17 HOMOLOGOUS POLYPEPTIDES AND THERAPEUTIC USES THEREOF****FIELD OF THE INVENTION**

The present invention relates generally to the identification and isolation of novel DNA, therapeutic uses and the recombinant production of novel polypeptides having  
10 sequence identity with the cytokine IL-17, and cytotoxic T-lymphocyte-associated antigen 8 (CTLA-8) designated herein as PRO1031 and PRO1122 polypeptides.

**BACKGROUND OF THE INVENTION**

It has been reported that the cytokine interleukin 17 (IL-17) stimulates epithelial,  
15 endothelial, and fibroblastic cells to secrete cytokines such as IL-6, IL-8, and granulocyte-colony-stimulating factor, as well as prostaglandin E2. While expression of IL-17 is restricted to activated T cells, the IL-17 receptor is widely expressed, a property consistent with the pleiotropic activities of IL-17. Moreover, it has been shown that when cultured in the presence of IL-17, fibroblasts could sustain proliferation of CD34+ preferential  
20 maturation into neutrophils. As a result, IL-17 could be an early potentiator or even maintainer of T cell-dependent inflammatory reaction and/or an element of the cytokine network that bridges the immune system to hematopoiesis. See, Yao, *et al.*, *J. Immunol.*, 155(12):5483-5486 (1995); Fossiez, *et al.*, *J. Exp. Med.*, 183(6):2593-2603 (1996); Kennedy, *et al.*, *J. Interferon Cytokine Res.*, 16(8):611-617 (1996).

25 More generally, all novel proteins are of interest. Extracellular proteins play an important role in the formation, differentiation and maintenance of multicellular organisms. The fate of many individual cells, e.g., proliferation, migration, differentiation, or interaction with other cells, is typically governed by information received from other cells and/or the immediate environment. This information is often transmitted by secreted polypeptides (for  
30 instance, mitogenic factors, survival factors, cytotoxic factors, differentiation factors, neuropeptides, and hormones) which are, in turn, received and interpreted by diverse cell receptors or membrane-bound proteins. These secreted polypeptides or signaling molecules normally pass through the cellular secretory pathway to reach their site of action in the extracellular environment.

35 Secreted proteins have various industrial applications, including pharmaceuticals, diagnostics, biosensors and bioreactors. Most protein drugs available at present, such as thrombolytic agents, interferons, interleukins, erythropoietins, colony stimulating factors, and various other cytokines, are secretory proteins. Their receptors, which are membrane proteins, also have potential as therapeutic or diagnostic agents.



Efforts are being undertaken by both industry and academia to identify new, native secreted proteins. Many efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel secreted proteins. Examples of screening methods and techniques are described in the literature [see, for example, Klein *et al.*, *Proc. Natl. Acad. Sci.*, 93:7108-7113 (1996); U.S. Patent No. 5,536,637]. The results of such efforts are presented herein.

Interleukin-17 is a recently described, T cell-derived cytokine, the biological functions of which are only beginning to be understood. Spriggs *et al.*, *J. Clin. Immunol.* 17: 366 (1997); Broxmeyer, H.E., *J. Exp. Med.* 183: 2411 (1996). When IL-17 was initially identified as a cDNA clone from a rodent T-cell lymphoma, it was recognized as having a sequence similar to an open reading frame from a primate herpesvirus, *Herpesvirus saimiri* Rouvier *et al.*, *J. Immunol.* 150: 5445 (1993), Yao *et al.*, *Immunity* 3: 811 (1995) [Yao-1], Fossiez *et al.*, *J. Exp. Med.* 183: 2593 (1996). Subsequently, it has been confirmed that this viral protein has many if not all of the immunostimulatory activities found for the host IL-17. Fleckenstein and Desrosiers, "*Herpesvirus saimiri* and *herpesvirus ateles*," In *The Herpesviruses*, I.B. Roizman, ed, Plenum Publishing Press, New York, p.253 (1982), Biesinger, B.I. *et al.*, *Proc Natl Acad Sci. USA* 89: 3116 (1992).

Human IL-17 is a 20-30 kDa, disulfide linked, homodimeric protein with variable glycosylation. Yao-1, *supra*; Fossiez *et al.*, *supra*. It is encoded by a 155 amino acid open reading frame that includes an N-terminal secretion signal sequence of 19-23 amino acids. The amino acid sequence of IL-17 is only similar to the *Herpesvirus* protein described above and does not show significant identity with the sequences of other cytokines or other known proteins. Additionally, the IL-17 encoding mRNA has been detected has only been detected in activated CD4<sup>+</sup> memory T cells and PMA/ionomycin stimulated PBMC cells.

Despite its restricted tissue distribution, IL-17 exhibits pleiotropic biological activities on various types of cells, such as fibroblasts, endothelial cells and epithelial cells. Spriggs, M.K., *supra*; Broxmeyer, H.E., *supra*. IL-17 has been found to stimulate the production of many cytokines: TNF- $\alpha$  and IL-1 $\beta$  from macrophages [Jovanovic *et al.*, *J. Immunol* 160: 3513 (1998)]; IL-6, IL-8 and the intracellular adhesion molecule (ICAM-1) from human fibroblasts. Fossiez *et al.*, *supra*, Yao *et al.*, *J. Immunol.* 155: 5483 (1995) [Yao-2]; granulocyte-colony-stimulating factor (G-CSM) and prostaglandin (PGE-2) from synoviocytes, Fossiez *et al.*, *supra*. Through the induction of a number of cytokines, IL-17 is able to mediate a wide-range of response, mostly proinflammatory and hematopoietic. This has led to the suggestion that IL-17 may play a pivotal role in initiating or sustaining an inflammatory response. Jovanovic *et al.*, *supra*.

Consistent with IL-17's wide-range of effects, the cell surface receptor for IL-17 has been found to be widely expressed in many tissues and cell types Yao *et al.*, *Cytokine* 9: 794 (1997) [Yao-3]. While the amino acid sequence of the hIL-17 receptor (866 a.a.) predicts a

protein with a single transmembrane domain and a long, 525 amino acid intracellular domain, the receptor sequence is unique and is not similar to that of any of the receptor from the cytokine/growth factor receptor family. This coupled with the lack of similarity of IL-17 itself to other known proteins indicates that IL-17 and its receptor may be part of a novel family of signaling proteins and receptors.

IL-17 has further been shown, by intracellular signaling, to stimulate transient  $\text{Ca}^{2+}$  influx and a reduction in [cAMP]; in human macrophages. Jovanovic *et al.*, *supra*. Fibroblasts and macrophages treated with IL-17 induce the activation of NF- $\kappa$ B, Yao-1, *supra*, Jovanovic *et al.*, *supra*, while macrophages treated with it activate NF- $\kappa$ B and mitogen-activated protein kinases. Shalom-Barek *et al.*, *J. Biol. Chem.* 273: 27467 (1998).

The present invention describes the cloning and characterization of two novel proteins, termed PRO1031 (IL-17B) and PRO1122 (IL-17C), and active variants thereof, that are similar in amino acid sequence to IL-17.

### SUMMARY OF THE INVENTION

Applicants have identified a cDNA clone that encodes a novel polypeptide having sequence identity with IL-17, wherein the polypeptide is designated in the present application as "PRO1031" or "PRO1122".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1031 or PRO1122 polypeptide.

In one aspect, the isolated nucleic acid comprises DNA encoding the PRO1031 or PRO1122 polypeptide having amino acid residues: from about 21 through 180 of Figure 1 (SEQ ID NO:1), or from about 19 through 197 of Figure 3 (SEQ ID NO:3), respectively, or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the isolated nucleic acid comprises DNA having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98%

sequence identity, yet more preferably at least about 99% sequence identity to (a) a DNA molecule encoding a PRO1031 or PRO1122 polypeptide comprising the sequence of amino acid residues from 1 or about 21 to 180, inclusive, of Figure 1 (SEQ ID NO:1) or from 1 or about 19 to 197, inclusive, of Figure 3 (SEQ ID NO:3), or the (b) the complement of the DNA molecule of (a). Alternatively, the isolated nucleic acid comprises DNA encoding the PRO1031 polypeptide having amino acid residues 1 through 180, inclusive, of Figure 3 (SEQ ID NO:3). Alternatively, the isolated nucleic acid comprises DNA encoding a 1122 polypeptide having the sequence of amino acid residues from about 1 to about 197, inclusive of Figure 1 (SEQ ID NO:1).

10 In another aspect, the invention concerns an isolated nucleic acid molecule encoding a PRO1031 or PRO1122 polypeptide comprising DNA hybridizing to the complement of the nucleic acid between about residues: (a) 42 to about 581, inclusive, of Figure 2 (SEQ ID NO:2), or (b) 49 to about 640, inclusive, or Figure 4 (SEQ ID NO:4), respectively. Preferably, the hybridization range extends from about nucleic acid residue (a) about 102 to about 581, inclusive, of Figure 2 (SEQ ID NO:2), or (b) about 104 to about 640, inclusive, of Figure 4 (SEQ ID NO:4), respectively. Preferably, hybridization occurs under stringent hybridization and wash conditions.

In another aspect, the invention concerns an isolated nucleic acid molecule encoding an active PRO1031 or PRO1122 polypeptide comprising a nucleotide sequence that hybridizes to the complement of a nucleic acid sequence that encodes amino acids (a) 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (b) 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3). Preferably, hybridization occurs under stringent hybridization and wash conditions.

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising DNA having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA in

ATCC deposit No. 209866 (DNA59294-1381) or 203552 (DNA62377-1381-1). In a preferred embodiment, the nucleic acid comprises DNA encoding the same mature polypeptide encoded by the human protein cDNA in ATCC deposit number 209866 (DNA59294-1381) or 203553 (DNA62377-1381-1), deposited on 14 May 1998 and 23  
 5 December 1998, respectively. In a more preferred embodiment, the nucleic acid comprises the cDNA insert of ATCC deposit DNA59294-1381 (ATCC 209866) deposited on 14 May 1998 or DNA62377-1381-1 (ATCC 203552), deposited on 22 December 1998, respectively.

In another aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein having at least about 80% sequence  
 10 identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88%  
 15 sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97%  
 20 sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) the full-length polypeptide encoded by the cDNA deposited with the ATCC on (1) 14 May 1998 under ATCC Deposit No.: 209866 (DNA59294-1381) or (2) 23 December 1998 under ATCC Deposit No.: 203553 (DNA62377-1381-1), or (b) the complement of the nucleotide sequence of (a). In a preferred  
 25 embodiment, the isolated nucleic acid molecule encodes the same full length polypeptide as the cDNA deposit of ATCC Deposit No.: 209866 or 203553, respectively.

In a further aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% sequence identity, preferably at least about 81% sequence  
 30 identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89%  
 35 sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94%

sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity, to: (a) DNA molecule comprising the  
 5 sequence of nucleotides from about 42 or about 102 to about 581, inclusive, of Figure 2 (SEQ ID NO:2) or from about 49 or about 104 to about 640, inclusive, of Figure 4 (SEQ ID NO:4); or (b) the complement of the DNA molecule of (a).

In another aspect, the isolated nucleic acid molecule comprises: (a) the nucleotide sequence from about 42 or about 102 to about 581, inclusive, of Figure 2 (SEQ ID NO:2) or  
 10 from about 49 or about 104 to about 640, inclusive, of Figure 4 (SEQ ID NO:4); or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule produced by hybridizing a test DNA molecule under stringent conditions with: (a) a DNA molecule encoding (i) a PRO1031 polypeptide having the sequence of amino acid residues from about  
 15 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (ii) a PRO1122 polypeptide having the sequence of amino acid residues from about 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3); or (b) the complement of the DNA molecule of (a), and if the DNA molecule has at least about an 80% sequence identity, preferably at least about an 81% sequence identity, more preferably at least about a 82% sequence identity, yet  
 20 more preferably at least about a 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90%  
 25 sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more  
 30 preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to (a) or (b), isolating the test DNA molecule.

In yet a further aspect, the invention concerns an isolated nucleic acid molecule comprising: (a) DNA encoding a polypeptide scoring at least about 80% positives, preferably at least about 81% positives, more preferably at least about 82% positives, yet more  
 35 preferably at least about 83% positives, yet more preferably at least about 84% positives, yet more preferably at least about 85% positives, yet more preferably at least about 86% positives, yet more preferably at least about 87% positives, yet more preferably at least about 88% positives, yet more preferably at least about 89% positives, yet more preferably at least

about 90% positives, yet more preferably at least about 91% positives, yet more preferably at least about 92% positives, yet more preferably at least about 93% positives, yet more preferably at least about 94% positives, yet more preferably at least about 95% positives, yet more preferably at least about 96% positives, yet more preferably at least about 97% positives, yet more preferably at least about 98% positives, yet more preferably at least about 99% positives, when compared with the amino acid sequence of residues about (i) 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (ii) 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3), or (b) the complement of the DNA of (a).

In a specific aspect, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO1032 or PRO1122 polypeptide without the N-terminal signal sequence and/or initiating methionine, or is complementary to such encoding nucleic acid molecule. The signal peptide has been tentatively identified as extending from about amino acid residue (a) 1 to about amino acid residue 20, inclusive, in the sequence of Figure 1 (SEQ ID NO:1), or (b) 1 to about amino acid residue 18, inclusive, in the sequence of Figure 3 (SEQ ID NO:3). It is noted, however, that the C-terminal boundary of the signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art. Nielsen *et al.*, *Prot. Engin.* 10: 1-6 (1997), von Heinje *et al.*, *Nucl. Acids Res.* 14: 4683-4690 (1986). Moreover, it is also recognized that, in some cases, cleavage of the signal sequence form a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding them, are contemplated by the present invention. A such, for purposed of the present application, the signal peptide of the PRO1032 or PRO1122 polypeptide shown in Figure 1 (SEQ ID NO:1) or Figure 3 (SEQ ID NO:3), respectively, extends from amino acids 1 to X, wherein X is any amino acid from (a) 15 to 25 of Figure 1 (SEQ ID NO:1), or (b) 13 to 23 of Figure 3 (SEQ ID NO:3), respectively of Figure 3.

Another embodiment is directed to fragments of a PRO1031- or PRO1122-encoding sequence that may find use as, for example, hybridization probes or for encoding fragments of a PRO1031 or PRO1122 polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO1031 or anti-PRO1122 antibody. Such nucleic acids fragments are usually at least about 20 nucleotides in length, preferably at least about 30 nucleotides in length, more preferable at least about 40 nucleotides in length, yet more preferably at least about 50 nucleotides in length, yet more preferably at least about 60 nucleotides in length, yet more preferably at least about 70 nucleotides in length, yet more preferably at least about 80 nucleotides in length, yet more preferably at least about 90 nucleotides in length, yet more preferably at least about 100 nucleotides in length, yet more preferably at least about 110 nucleotides in length, yet more preferably at least about 120 nucleotides in length, yet more

preferably at least about 130 nucleotides in length, yet more preferably at least about 140 nucleotides in length, yet more preferably at least about 150 nucleotides in length, yet more preferably at least about 160 nucleotides in length, yet more preferably at least about 170 nucleotides in length, yet more preferably at least about 180 nucleotides in length, yet more preferably at least about 190 nucleotides in length, yet more preferably at least about 200 nucleotides in length, yet more preferably at least about 250 nucleotides in length, yet more preferably at least about 300 nucleotides in length, yet more preferably at least about 350 nucleotides in length, yet more preferably at least about 400 nucleotides in length, yet more preferably at least about 450 nucleotides in length, yet more preferably at least about 500 nucleotides in length, yet more preferably at least about 600 nucleotides in length, yet more preferably at least about 700 nucleotides in length, yet more preferably at least about 800 nucleotides in length, yet more preferably at least about 900 nucleotides in length, yet more preferably at least about 1000 nucleotides in length, wherein in this context "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. In a preferred embodiment, the nucleotide sequence fragment is derived from any coding region of the nucleotide sequence shown in Figure 2 (SEQ ID NO:2) or Figure 4 (SEQ ID NO:4). In a more preferred embodiment, the nucleotide sequence fragment is derived from nucleotides about 50 to about 390 and about 621 through about 640, inclusive, of Figure 4 (SEQ ID NO:4). Alternatively, the nucleotide sequence fragment can be derived from a fragment within the region between 391 and 620, inclusive, provided at least one nucleotide is included outside of the region (*i.e.*, 50-390, 621-640).

In another embodiment, the invention provides a vector comprising DNA encoding a PRO1031 or PRO1122 or its variants. The vector may comprise any of the isolated nucleic acid molecules hereinabove defined.

In another embodiment, the invention provides a host cell comprising the above vector. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing PRO1031 or PRO1122 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of PRO1031 or PRO1122, respectively, and recovering PRO1031 or PRO1122, respectively, from the cell culture.

In another embodiment, the invention provides isolated PRO1031 or PRO1122 polypeptides encoded by any of the isolated nucleic acid sequences hereinabove defined.

In another aspect, the invention concerns an isolated PRO1031 or PRO1122 polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more

preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to the sequence of amino acid residues about (a) 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (b) 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3), respectively. In a preferred aspect, the polypeptide comprises amino acid residues about (a) 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1) or (b) 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3), respectively.

In a further aspect, the invention concerns an isolated PRO1031 or PRO1122 polypeptide comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% sequence identity to the amino acid encoded by the human protein cDNA deposited with the ATCC on (1) 14 May 1999 under ATCC Deposit No. 209866 (DNA59294-1381) or (2) 22 December 1998 under ATCC Deposit No. 203552, respectively.

In a preferred embodiment, the PRO1031 or PRO1122 polypeptide is obtained or obtainable by expressing the polypeptide encoded by the cDNA insert of the vector deposited on (a) 14 May 1998 under ATCC deposit number 209866 (DNA59294-1381), or (b) 22 December 1998 under ATCC deposit number 203552 (DNA62377-1381-1).

In a further aspect, the invention concerns an isolated PRO1031 or PRO1122 polypeptide comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 81% positives, more preferably at least about 82% positives, yet more preferably at least about 83% positive, yet more preferably at least about 84% positives, yet more preferably at least about 85% positives, yet more preferably at least about 86%



positives, yet more preferably at least about 87% positives, yet more preferably at least about 88% positives, yet more preferably at least about 89% positives, yet more preferably at least about 90% positives, yet more preferably at least about 91% positives, yet more preferably at least about 92% positives, yet more preferably at least about 93% positives, yet more preferably at least about 94% positives, yet more preferably at least about 95% positives, yet more preferably at least about 96% positives, yet more preferably at least about 97% positives, yet more preferably at least about 98% positives, yet more preferably at least about 99% positives, when compared with the amino acid sequence of residues from about (1) 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (2) 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3).

In a specific aspect, the invention provides an isolated PRO1031 or PRO1122 polypeptide without the N-terminal signal sequence and/or initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as hereinbefore described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO1031 or PRO1122 polypeptide and recovering the PRO1031 or PRO1122 polypeptide, respectively, from the cell culture.

In still a further aspect, the invention provides a polypeptide produced by: (1) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a (i) PRO1031 polypeptide having the sequence of amino acid residues from about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (ii) PRO1122 polypeptide having the sequence of amino acid residues from about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3), or (b) the complement of the DNA molecule of (a); and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 81% sequence identity, more preferably at least about an 82% sequence identity, yet more preferably at least about an 83% sequence identity, yet more preferably at least about an 84% sequence identity, yet more preferably at least about an 85% sequence identity, yet more preferably at least about an 86% sequence identity, yet more preferably at least about an 87% sequence identity, yet more preferably at least about an 88% sequence identity, yet more preferably at least about an 89% sequence identity, yet more preferably at least about a 90% sequence identity, yet more preferably at least about a 91% sequence identity, yet more preferably at least about a 92% sequence identity, yet more preferably at least about a 93% sequence identity, yet more preferably at least about a 94% sequence identity, yet more preferably at least about a 95% sequence identity, yet more preferably at least about a 96% sequence identity, yet more preferably at least about a 97% sequence identity, yet more preferably at least about a 98% sequence identity, yet more preferably at least about a 99% sequence identity to (a) or (b); (2)

culturing a host cell comprising the test DNA molecule under conditions suitable for expression of the polypeptide, and (3) recovering the polypeptide from the cell culture.

In yet another aspect, the invention concerns an isolated PRO1031 or PRO1122 polypeptide comprising the sequence of amino acid residues from about (1) 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1), or (2) 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3), respectively, or a fragment thereof which is biologically active or sufficient to provide a binding site for an anti-PRO1031 or anti-PRO1122 antibody, respectively, wherein the identification of PRO1031 or PRO1122 polypeptide fragments, respectively, that possess biological activity or provide a binding site for an anti-PRO1031 or anti-PRO1122 antibody, respectively, may be accomplished in a routine manner using techniques which are well known in the art.

In another embodiment, the invention provides chimeric molecules comprising a PRO1031 or PRO1122 polypeptide fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises a PRO1031 or PRO1122 polypeptide, respectively, fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to a PRO1031 or PRO1122 polypeptide. Optionally, the antibody is a monoclonal antibody, an antibody fragment or a single chain antibody.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO1031 or PRO1122 polypeptide. In a particular aspect, the agonist or antagonist is an anti-PRO1031 or anti-PRO1122 antibody, or a small molecule.

In yet another embodiment, the invention concerns a method of identifying agonists or antagonists of a native PRO1031 or native PRO1122 polypeptide, by contacting the native PRO1031 or PRO1122 polypeptide with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In still a further embodiment, the invention concerns a composition comprising a PRO1031 or PRO1122 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a carrier. Preferably, the carrier is pharmaceutically acceptable.

In still a further embodiment, the invention concerns the use of a PRO1031 or PRO1122 polypeptide, or an agonist or antagonist thereof as hereinbefore described, or an anti-PRO1031 or anti-PRO1122 antibody, for the preparation of a medicament useful in the treatment of a condition which is responsive to the PRO1031 or PRO1122 polypeptide or an agonist or antagonist thereof (e.g., anti-PRO1031 or PRO1122). In a particular aspect, the invention concerns the use of a PRO1031 or PRO1122 polypeptide, or an agonist or antagonist thereof in a method for treating a degenerative cartilaginous disorder.

In still a further embodiment, the invention relates to a method of treating a degenerative cartilaginous disorder by administration of a therapeutically effective amount of

a PRO1031 or PRO1122 polypeptide, agonist, or antagonist thereof to a mammal suffering from said disorder.

In still a further embodiment, the invention relates to a method of diagnosing a degenerative cartilaginous disorder by (1) culturing test cells or tissues expressing PRO1031 or PRO1122; (2) administering a compound which can inhibit PRO1031 or PRO1122 modulated signaling; and (3) measuring the PRO1031 or PRO1122 mediated phenotypic effects in the test cells.

In still a further embodiment, the invention relates to PRO1031 or PRO1122 antagonists and/or agonist molecules. In one aspect, the inventions provides a method of screening compounds which mimic PRO1031 or PRO1122 (agonists) or diminish the effect of the PRO1031 or PRO1122 (antagonists).

In still a further embodiment, the invention relates to a therapeutic composition comprising a therapeutically effective amount of PRO1031, PRO1122, antagonist or agonist thereof in combination with a pharmaceutically-acceptable carrier.

In still a further embodiment, the invention relates to an article of manufacture comprising a container, label and therapeutically effective amount of PRO1031, PRO1122, antagonist or agonist thereof in combination with a pharmaceutically-acceptable carrier.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence (SEQ ID NO:1) derived from nucleotides 42-581 of SEQ ID NO:2. Also shown in Figure 1 are the signal peptide, an N-glycosylation site, a region having sequence identity with IL-17, the molecular weight, and approximate pI:

Figure 2 shows a nucleotide sequence (SEQ ID NO:2) containing the nucleotide sequence of a native sequence PRO1031 cDNA (nucleotides 42-581 of SEQ ID NO:2), wherein the nucleotide sequence (SEQ ID NO:2) is a clone designated herein as "UNQ516" and/or "DNA59294-1381".

Figure 3 shows the amino acid sequence (SEQ ID NO:3) derived from nucleotides 50-640 of SEQ ID NO:4. Also shown are the approximate locations of the signal peptide, a leucine zipper pattern in a region having sequence identity with IL-17. The approximate weight in daltons, and approximate pI are also shown.

Figure 4 shows a nucleotide sequence (SEQ ID NO:4) containing the nucleotide sequence of a native sequence PRO1122 cDNA (nucleotides 50-640 of SEQ ID NO:1), wherein the nucleotide sequence (SEQ ID NO:4) is a clone designated herein as "UNQ561" and/or "DNA62377-1381-1". The complementary sequence and deduced amino acid sequences are also shown.

Figure 5 shows DNA47332 (SEQ ID NO:5), a virtual DNA fragments used in the isolation of DNA59294 (SEQ ID NO:2).

Figure 6 shows DNA49665 (Incyte EST 1347523) (SEQ ID NO:7), a virtual fragment used in the isolation of DNA62377 (SEQ ID NO:4).

Figure 7A shows an alignment between the protein sequences encoded by DNA59624 (IL17-B)(SEQ ID NO:1), DNA62377 (IL17-C)(SEQ ID NO:3) and IL-17 (SEQ ID NO:11).  
 5 The putative signal sequences are underlined, potential N-linked glycosylation sites are double underlined, and conserved tryptophan and cysteine residues are marked with asterisks. IL-17, IL-17B and IL-17C share 26-28% amino acid identity with each other. Figure 7B shows an alignment between just the encoded protein from DNA59624 (SEQ ID NO:1) and DNA62377 (SEQ ID NO:3).

10 Figure 8 is an RNA blot analysis of IL-17B (UNQ516)(SEQ ID NO:1). The northern blot depicts mRNA from human tissues (Clontech) hybridized to a human IL17B specific radiolabeled probe as described in Example 8. RNA size markers are shown on the left. A rehybridization of the same blot with a human  $\beta$ -actin cDNA probe is shown at the bottom.

Figures 9A-9B depict bar graphs representing the biological activities of IL17 (SEQ ID NO:11), IL17B (UNQ516)(SEQ ID NO:1) and IL17C (UNQ561)(SEQ ID NO:3). Figure 9A shows human foreskin fibroblast (HFF) cells cultured with control Fc fusion protein, IL-17, IL-17B.Fc (SEQ ID NO:12) or IL-17C.Fc (SEQ ID NO:13) at 100 ng/ml for 18 hours and the conditioned media were assayed for IL-6 (SEQ ID NO:14) as described in Example 10. Figure 9B shows the human leukemic cell line, THP1, which was treated with the same  
 20 cytokines (100 ng/ml) as above under the same conditions wherein the supernatants were assayed for the level of TNF- $\alpha$  release. Results are expressed as the mean  $\pm$  SE of triplicate determinations from one representative experiment.

Figure 10 is a time course representing the dependence of IL17B and IL17C activated TNF- $\alpha$  release from THP1 cells. In Figure 10A, THP1 cells were incubated with 100 ng/ml  
 25 (2.2 nM) of IL17B.Fc (SEQ ID NO:12) or IL17C.Fc (SEQ ID NO:13) for 0.5 to 32 hours, the conditioned media harvested, and the TNF- $\alpha$  concentration quantitated as described in Example 10. In Figure 10B, THP1 cells were treated with the IL-17B.Fc and IL-17C.Fc at a concentration range from 0 to 120 nM for 18 hours and the TNF- $\alpha$  release determined.

Figure 11 is an immunoprecipitation of IL-17R ECD (SEQ ID NO:15) with IL-17  
 30 (SEQ ID NO:11), IL17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3). His-tagged IL-17 receptor ECD was expressed in 293 cells and metabolically labeled with  $^{35}$ S as described in Example 11. The supernatant was recovered and Ni-NTA beads were used to affinity precipitate the his-tagged IL-17R ECD (SEQ ID NO:15) in the supernatant (lane 1). In Figure 11A, IL-17 (SEQ ID NO:11), IL-17B.Fc (SEQ ID NO:12) and IL-17C.Fc (SEQ ID NO:13), or control Fc fusion proteins were incubated with the supernatant and protein-A-  
 35 agarose beads were added to precipitate the Fc fusion proteins. For the IL-17 immunoprecipitation reaction, anti-IL-17 antibodies were included. Figure 11B shows the results of a competitive binding experiment, wherein immunoprecipitation of IL-17R ECD

(SEQ ID NO:22) by IL-17 (SEQ ID NO:11) was performed in the presence of a five-fold excess of IL-17B.his (SEQ ID NO:23) and control his-tagged proteins. Precipitates in both Figure 11A and Figure 11B were analyzed by electrophoresis on NuPAGE (4-12% Bis-Tris) gels. Molecular weight markers are indicated on the left of each panel.

5 Figure 12 shows FACS analysis of the binding of IL-17B.Fc (SEQ ID NO:12) and IL-17C.Fc (SEQ ID NO:13) to THP-1 cells. THP-1 cells were incubated with IL-17B.Fc (A) or IL-17C.Fc (B) or control Fc fusion proteins in PBS (5% horse serum) and followed by addition of FITC conjugated anti-Fc secondary antibodies.

10 Figure 13 shows the effect of IL-17 (SEQ ID NO:11) on articular cartilage. Cartilage explants were cultured with the indicated concentration of IL-17 alone (solid) or in the presence of IL-1 $\alpha$  at the indicated concentration (hatched)(SEQ ID NO:25) or IL1ra (IL-1 receptor antagonist, R&D Systems, 1  $\mu$ g/ml)(SEQ ID NO:26) for 72 hours. Release of proteoglycans (PG) into the media (top panel) indicates matrix breakdown. Matrix synthesis was determined by incorporation of <sup>35</sup>S-sulphate into the tissue (bottom panel).

15 Figure 14 shows the effect of IL-17 (SEQ ID NO:11) on the release of nitric oxide. Explants were treated with IL-17 (10 ng/ml) alone (left columns) or in the presence of IL-1 $\alpha$  (10 ng/ml)(SEQ ID NO:25)(right columns). After 48 hours, media was assayed for nitrite concentration.

20 Figure 15 shows the effect of NO on IL-17 induced changes in matrix metabolism. Explants were treated with IL-17 (5 ng/ml)(SEQ ID NO:11) alone (+) or with an irreversible inhibitor of nitric oxide synthase, NOS (L-NIO, Caymen Chemical, 0.5mM). After 72 hours of treatment, media was assayed for (A) nitrite and (B) proteoglycans (PGs). (C) Proteoglycan synthesis was determined by incorporation of <sup>35</sup>S-sulphate into the tissue.

25 Figure 16 shows the effect of the inhibition of NO on IL-1 $\alpha$ -induced changes in proteoglycan (PG) metabolism. Articular cartilage explants were treated with IL-1 $\alpha$  (5 ng/ml)(SEQ ID NO:25) alone (+) or with inhibitors of NOS (L-NIO or L-NIL) (L-NIL, reversible NOS inhibitor, Caymen Chemical) or IL-1ra (IL-1 receptor antagonist, R&D Systems, 1  $\mu$ g/ml)(SEQ ID NO:26). After 72 hours of treatment, media was assayed for (A) nitrite concentration and (B) amount of proteoglycans. (C) Matrix synthesis was determined  
30 by incorporation of <sup>35</sup>S-sulphate into the tissue.

Figure 17 shows the effect of UNQ516 (SEQ ID NO:1) on articular cartilage. Explants were treated with UNQ561 at 1% or 0.1% in the absence (leftmost 3 columns) or presence (rightmost three columns) of IL-1 $\alpha$  (SEQ ID NO:25) at 10 ng/ml, and proteoglycan (PG) synthesis and nitrite production were determined as described in Example 16.

35 Figure 18 shows the effect of UNQ561 (SEQ ID NO:3) on articular cartilage. Explants were treated with UNQ561 at 1% or 0.1% in the absence (leftmost three columns) or presence (rightmost three columns) of IL-1 $\alpha$  (+) (10 ng/ml)(SEQ ID NO:25). Proteoglycan (PG) release and synthesis are shown as amount above control.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### I. Definitions

The terms "PRO1031 polypeptide", or "PRO1122 polypeptide" and "PRO1031", or "PRO1122" when used herein encompass native sequence PRO1031, native sequence PRO1122, respectively and polypeptide variants thereof (which are further defined herein). The PRO1031 or PRO1122 polypeptides may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A "native sequence PRO1031 polypeptide" or "native sequence PRO1122 polypeptide" comprise a polypeptide having the same amino acid sequence as a PRO1031 or PRO1122 polypeptide, respectively, derived from nature. Such native sequence PRO1031 or PRO1122 polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO1031 polypeptide" or "native sequence PRO1122 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of a PRO1031 polypeptide or PRO1122 polypeptide, respectively, (*e.g.*, soluble forms containing for instance, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of a PRO1031 or PRO1122 polypeptide, respectively.

In one embodiment of the invention, the native sequence PRO1031 polypeptide or PRO1122 polypeptide is a full-length or mature native sequence (a) PRO1031 polypeptide comprising amino acids 1 or 21 through 180 of Figure 1 (SEQ ID NO:1) or (b) PRO1122 polypeptide comprising amino acids 1 or 19 through 197 of Figure 3 (SEQ ID NO:3), respectively. Also, while the PRO1031 or PRO1122 polypeptides disclosed in Figures 1 and 3, respectively, (*i.e.*, UNQ516 and UNQ561), are shown to begin with a methionine residue designated as amino acid position 1, it is conceivable and possible that another methionine residue located either upstream or downstream from amino acid position 1 in Figure 1 or Figure 3 may be employed as the starting amino acid residue.

The term "UNQ516" or "UNQ561" refer to the specific native sequence PRO1031 or PRO1122 protein, respectively, depicted in Figure 1 or 3, respectively. Optionally, the PRO1031 or PRO1122 polypeptide is obtained or obtainable by expressing the polypeptide encoded by the cDNA insert of the vector DNA59294-1381 or DNA62377-1381-1, under ATCC deposit number 209866 or 203552, respectively.

"PRO1031 variant" or "PRO1122 variant" means an "active" PRO1031 polypeptide or PRO1122 polypeptide, respectively, as defined below having at least about 80% amino acid sequence identity with the PRO1031 polypeptide or PRO1122 polypeptide, respectively, having the deduced amino acid sequence of residues (1) 1 or about 21 to about 180 shown in Figure 1 (SEQ ID NO:1), or (2) 1 or about 19 to 197 shown in Figure 3 (SEQ ID NO:3), respectively, for a full-length or mature native sequence PRO1031 or PRO1122 polypeptide,

respectively. Such PRO1031 or PRO1122 polypeptide variants include, for instance, PRO1031 polypeptides or PRO1122 polypeptides, respectively, wherein one or more amino acid residues are added, substituted or deleted, at the N- or C-terminus or within the sequence of Figure 1 (SEQ ID NO:1) or Figure 3 (SEQ ID NO:3), respectively. Ordinarily, a  
 5 PRO1031 or PRO1122 polypeptide variant will have at least about 80% amino acid sequence identity, preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity,  
 10 yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97%  
 15 sequence identity, yet more preferably at least about 98% sequence identity, yet more preferably at least about 99% amino acid sequence identity with the amino acid sequence of Figure 1 (SEQ ID NO:1) or Figure 3 (SEQ ID NO:3), with or without the signal peptide (E.g., with signal peptide amino acid residues 1 to 180 of SEQ ID NO:1, 1 to 197 of SEQ ID NO:3, without signal peptide about 21 to 180 of SEQ ID NO:1, about 19 to 197 of SEQ ID  
 20 NO:3). The variants provided herein exclude native sequence PRO1031 and PRO1122 sequences as well the polypeptides and nucleic acids described herein with which the PRO1031 and PRO1122 polypeptides share 100% identity and/or which are already known in the art.

"Percent (%) amino acid sequence identity" with respect to the PRO1031 amino acid  
 25 sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a PRO1031 polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence  
 30 identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, ALIGN-2, Megalign (DNASTAR) or BLAST (e.g., Blast, Blast-2, WU-Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, the  
 35 % identity values used herein are generated using WU-BLAST-2 (Altschul *et al.*, *Methods in Enzymology* 266: 460-480 (1996). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with

the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM 62. For purposes herein, a % amino acid sequence identity value is determined by divided (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO1031 or PRO1122 polypeptide of interest and the comparison amino acid sequence of interest (*i.e.*, the sequence against which the PRO1031 or PRO1122 polypeptide of interest is being compared) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO1031 or PRO1122 polypeptide of interest, respectively.

A "PRO1031 or PRO1122 variant polynucleotide" or PRO1031 or PRO1122 variant nucleic acid sequence" means an active PRO1031 or PRO1122 polypeptide-encoding nucleic acid molecule as defined below having at least about 65% nucleic acid sequence identity with the nucleotide acid sequence of nucleotides: (1) about 42 or about 102 to about 589 or about 687 of the PRO1031-encoding nucleotide sequence shown in Figure 2 (SEQ ID NO:2); or (2) about 59 or about 104 to about 640 or about 1043 of the PRO1122-encoding nucleotide sequence shown in Figure 4 (SEQ ID NO:4), respectively. Ordinarily, a PRO1031 or PRO1122 polypeptide will have at least about 65% nucleic acid sequence identity, more preferably at least about 70% nucleic acid sequence identity, yet more preferably at least about 75% nucleic acid sequence identity, yet more preferably at least about 80% nucleic acid sequence identity, yet more preferably at least about 81% nucleic acid sequence identity, yet more preferably at least about 82% nucleic acid sequence identity, yet more preferably at least about 83% nucleic acid sequence identity, yet more preferably at least about 84% nucleic acid sequence identity, yet more preferably at least about 85% nucleic acid sequence identity, yet more preferably at least about 86% nucleic acid sequence identity, yet more preferably at least about 87% nucleic acid sequence identity, yet more preferably at least about 88% nucleic acid sequence identity, yet more preferably at least about 89% nucleic acid sequence identity, yet more preferably at least about 90% nucleic acid sequence identity, yet more preferably at least about 91% nucleic acid sequence identity, yet more preferably at least about 92% nucleic acid sequence identity, yet more preferably at least about 93% nucleic acid sequence identity, yet more preferably at least about 94% nucleic acid sequence identity, yet more preferably at least about 95% nucleic acid sequence identity, yet more preferably at least about 96% nucleic acid sequence identity, yet more preferably at least about 97% nucleic acid sequence identity, yet more preferably at least about 98% nucleic acid sequence identity, yet more preferably at least about 99% nucleic acid sequence identity with the nucleic acid sequence of nucleotides: 1) about 42 or about 102 to about 589 of the



PRO1031-encoding nucleotide sequence shown in Figure 2 (SEQ ID NO:2); or (2) about 59 or about 104 to about 640 of the PRO1122-encoding nucleotide sequence shown in Figure 4 (SEQ ID NO:4), respectively. Variants specifically exclude or do not encompass the native nucleotide sequence, as well as those prior art sequences which share 100% identity with the nucleotide sequences of the invention.

"Percent (%) nucleic acid sequence identity" with respect to the PRO1031 or PRO1122 sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO1031 sequence or PRO1122 sequence, respectively, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as ALIGN, Align-2, Megalign (DNASTAR), or BLAST (e.g., Blast, Blast-2) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % nucleic acid identity values are generated using the WU-BLAST-2 (BlastN module) computer program (Altschul *et al.*, *Methods in Enzymology* 266: 460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set default values, *i.e.*, the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11 and scoring matrix = BLOSUM62. For purposes herein, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest and the comparison nucleic acid molecule of interest (*i.e.*, the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest.

In other embodiments, the PRO1031 or PRO1122 variant polypeptides are nucleic acid molecules that encode an active PRO1031 or PRO1122 polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding the full-length PRO1031 or PRO1122 polypeptide shown in Figure 2 (SEQ ID NO:2) or Figure 4 (SEQ ID NO:4), respectively. This scope of variant polynucleotides specifically excludes those sequences which are known as of the filing and/or priority dates of the present application. Furthermore, PRO1031 or PRO1122 variant

polypeptides may also be those that are encoded by a PRO1031 or PRO1122 variant polynucleotide, respectively.

The term "positives", in the context of sequence comparison performed as described above, includes residues in the sequences compared that are not identical but have similar properties (e.g., as a result of conservative substitutions). The % identity value of positives is determined by the fraction of residues scoring a positive value in the BLOSUM 62 matrix. This value is determined by dividing (a) the number of amino acid residues scoring a positive value in the BLOSUM62 matrix of WU-BLAST-2 between the PRO1031 or PRO1122 polypeptide amino acid sequence of interest and the comparison amino acid sequence (*i.e.*, the amino acid sequence against which the PRO1031 or PRO1122 polypeptide sequence is being compared) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO1031 or PRO1122 polypeptide of interest.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO1031 or PRO1122 polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO1031 or PRO1122 polypeptide-encoding nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the PRO1031 polypeptide- or PRO1122 polypeptide-encoding nucleic acid. An isolated PRO1031 polypeptide- or PRO1122 polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated PRO1031 polypeptide- or PRO1122 polypeptide-encoding nucleic acid molecules therefore are distinguished from the PRO1031 polypeptide- or PRO1122 polypeptide-, respectively, encoding nucleic acid molecule as it exists in natural cells. However, an isolated PRO1031 polypeptide- or PRO1122 polypeptide-encoding nucleic acid molecule includes PRO1031 polypeptide- or PRO1122 polypeptide-, respectively, encoding nucleic acid molecules contained in cells that ordinarily

express PRO1031 polypeptide or PRO1122 polypeptide, where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator  
5 sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in  
10 the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the  
15 case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Stringency" of hybridization reactions is readily determinable by one of ordinary  
20 skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes required higher temperatures for proper annealing, while short probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The  
25 higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature that can be used. As a result, it follows that higher relative temperatures would tend to make the reactions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers,  
30 (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that" (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example,  
35 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M

NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash  
5 consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (*e.g.*, temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately  
10 stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, *etc.* as necessary  
15 to accommodate factors such as probe length and the like.

The term "epitope tagged" where used herein refers to a chimeric polypeptide comprising a PRO1031 or PRO1122 polypeptide, or domain sequence thereof, fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody may be made, or which can be identified by some other agent, yet is short  
20 enough such that it does not interfere with the activity of the PRO1031 or PRO1122 polypeptide. The tag polypeptide preferably is also fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 to about 50 amino acid residues (preferably, between about 10 to about 20 residues).

25 As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesion") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an  
30 immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin

may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA (including IgG-1 and IgA-2), IgE, IgD or IgM.

The term "antibody" is used in the broadest sense and specifically covers single anti-PRO1031 or anti-PRO1122 polypeptide monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO1031 or anti-PRO1122, respectively, antibody compositions with polyepitopic specificity, single-chain anti-PRO1031 or anti-PRO1122 antibodies, and fragments of anti-PRO1031 or anti-PRO1122 antibodies. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Active" or "activity" for the purposes herein refers to form(s) of PRO1031 or PRO1122 which retain the biologic and/or immunologic activities of native or naturally-occurring PRO1031 or PRO1122, respectively, polypeptide. Elaborating further, "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO1031 or PRO1122 other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO1031 or PRO1122 and an "immunological" activity refers only to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO1031 or PRO1122. A preferred biological activity includes, for example, the release of TNF- $\alpha$  from THP1 cells. An alternative activity is the reduction in IL-1 $\alpha$  induced NO (nitric oxide) production from articular cartilage.

"Degenerative cartilagenous disorder" describes a host of disorders that is characterized principally by the destruction of the cartilage matrix. Additional pathologies includes nitric oxide production, and elevated proteoglycan breakdown. Exemplary disorders encompassed within this definition, include, for example, arthritis (*e.g.*, osteoarthritis, rheumatoid arthritis, psoriatic arthritis), sepsis, ulcerative colitis, psoriasis, multiple sclerosis, type I diabetes, giant cell arthritis, systemic lupus erythematosus and Sjögren's syndrome.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO1031 or PRO1122 polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO1031 or PRO1122 polypeptide disclosed herein. Suitable agonist or antagonist

molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO1031 or PRO1122 polypeptides, peptides, small organic molecules, *etc.* Method for identifying agonists or antagonists of a PRO1031 or PRO1122 polypeptide may comprise contacting a PRO1031 or PRO1122 polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO1031 or PRO1122 polypeptide.

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is used in the broadest sense and specifically covers, without limitation, intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

The terms "treating", "treatment" and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy. An example of "preventative therapy" is the prevention or lessened targeted pathological condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, domestic and farm animals, and zoo, sports or pet animals, such as cattle (*e.g.* cows), horses, dogs, sheep, pigs, rabbits, goats, cats, *etc.* In a preferred embodiment of the invention, the mammal is a human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

A "therapeutically-effective amount" is the minimal amount of active agent (*e.g.*, PRO1031, PRO1122, antagonist or agonist thereof) which is necessary to impart therapeutic benefit to a mammal. For example a "therapeutically-effective amount" to a mammal

suffering or prone to suffering or to prevent it from suffering from a degenerative cartilagenous disorder is such an amount which induces, ameliorates or otherwise causes an improvement in the pathological symptoms, disease progression, physiological conditions associated with or resistance to succumbing to a disorder principally characterized by the  
5 destruction of the cartilage matrix.

"Carriers" as used herein include pharmaceutically-acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically-acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as  
10 phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecule weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating  
15 agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>®</sup>, polyethylene glycol (PEG), and PLURONICS<sup>™</sup>.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include  
20 Fab, Fab', F(ab')<sub>2</sub> and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Engin.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment,  
25 a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the  
30 three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fv fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domain which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097, WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues



of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue, or preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not  
 5 be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (*e.g.*, radioisotope labels or fluorescent  
 10 labels) or, in the case of an enzymatic label, may catalyze chemical alternation of a substrate compound or composition which is detectable.

"Solid phase" is meant to be a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*,  
 15 agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

20 A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO1031 or PRO1122 polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

25 A "small molecule" is defined herein to have a molecule weight below about 500 Daltons.

The term "modulate" means to affect (*e.g.*, either upregulate, downregulate or otherwise control) the level of a signaling pathway. Cellular processes under the control of signal transduction include, but are not limited to, transcription of specific genes, normal  
 30 cellular functions, such as metabolism, proliferation, differentiation, adhesion, apoptosis and survival, as well as abnormal processes, such as transformation, blocking of differentiation and metastasis.

## II. Compositions and Methods of the Invention

### A. Full-length PRO1031 or PRO1122 Polypeptide

The present invention provides newly identified and isolated nucleotide sequences  
5 encoding polypeptides referred to in the present application as PRO1031 or PRO1122. In particular, Applicants have identified and isolated cDNA encoding a PRO1031 (e.g., UNQ516, IL-17B, SEQ ID NO:1) and PRO1122 (e.g., UNQ561, IL-17C, SEQ ID NO:3) polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that various portions of the  
10 PRO1031 and PRO1122 polypeptide have sequence identity with IL-17. Accordingly, it is presently believed that PRO1031 and PRO1122 polypeptide disclosed in the present application are newly identified members of the cytokine family and thus may be involved in inflammation and/or the immune system function.

As presented earlier, the term "PRO1031" or "PRO1122" refers to the native sequence  
15 and variants, whereas the terms "UNQ516" or "UNQ561" refer to the specific amino acid sequences of Figure 1 (SEQ ID NO:1) and Figure 3 (SEQ ID NO:3), respectively, and/or the proteins encoded by the cDNA deposited with the American Type Culture Collection, under Deposit numbers 209866 and 203552, respectively.

As disclosed in the Examples below, cDNA clone designated herein as DNA59294-  
20 1381 and DNA62377-1381-1 have been deposited with the ATCC. The actual nucleotide sequence of the clone can be readily determined by the skilled artisan by sequencing of the deposited clone using routine methods in the art. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO1031 or PRO1122 polypeptide and encoding nucleic acid described herein, Applicants have identified what is  
25 believed to be the reading frame best identifiable with the sequence information available at the time.

### B. PRO1031 and PRO1122 Variants

In addition to the full-length native sequence PRO1031 or PRO1122 polypeptide  
30 described herein, it is contemplated that PRO1031 or PRO1122 variants can be prepared. PRO1031 or PRO1122 variants can be prepared by introducing appropriate nucleotide changes into the PRO1031- or PRO1122-encoding DNA, or by synthesis of the desired PRO1031 or PRO1122 polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO1031 or PRO1122 polypeptide,

such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO1031 or PRO1122 or in various domains of the PRO1031 or PRO1122 polypeptide described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO1031 or PRO1122 polypeptide that results in a change in the amino acid sequence of the PRO1031 or PRO1122 polypeptide as compared with the native sequence PRO1031 or PRO1122. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO1031 or PRO1122 polypeptide. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO1031 or PRO1122 polypeptide with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity (such as in any of the *in vitro* assays described in the Examples below) for activity exhibited by the full-length or mature native sequence.

PRO1031 or PRO1122 polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length or native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO1031 or PRO1122 polypeptide.

PRO1031 or PRO1122 fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO1031 or PRO1122 fragments by enzymatic digestion, *e.g.*, by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by

polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO1031 or PRO1122 polypeptide fragments share at least one biological and/or immunological activity with the native PRO1031 or PRO1122 polypeptide shown in Figure 1 (SEQ ID NO:1) or Figure 3 (SEQ ID NO:3).

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

**Table 1**  
**Conservative Substitutions**

| Original residue | Example substitutions                  | Preferred substitutions |
|------------------|--|-------------------------|
| Ala (A)          | val, leu, ile                          | val                     |
| Arg (R)          | lys, gln, asn                          | lys                     |
| Asn (N)          | gln, his, lys, arg                     | gln                     |
| Asp (D)          | glu                                    | glu                     |
| Cys (C)          | ser                                    | ser                     |
| Gln (Q)          | asn                                    | asn                     |
| Glu (E)          | asp                                    | asp                     |
| Gly (G)          | pro, ala                               | ala                     |
| His (H)          | asn, gln, lys, arg                     | arg                     |
| Ile (I)          | leu, val, met, ala, phe,<br>norleucine | leu                     |
| Leu (L)          | norleucine, ile, val, met, ala,<br>phe | ile                     |
| Lys (K)          | arg, gln, asn                          | arg                     |
| Met (M)          | leu, phe, ile                          | leu                     |
| Phe (F)          | leu, val, ile, ala, tyr                | leu                     |
| Pro (P)          | ala                                    | ala                     |
| Ser (S)          | thr                                    | thr                     |
| Thr (T)          | ser                                    | ser                     |
| Trp (W)          | tyr, phe                               | tyr                     |

|         |  |     |
|---------|--|-----|
| Tyr (Y) | trp, phe, thr, ser                     | phe |
| Val (V) | ile, leu, met, phe, ala,<br>norleucine | leu |

Substantial modifications in function or immunological identity of the PRO1031 or PRO1122 polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: sys, ser, thr;
- (2) neutral hydrophilic: cys, ser, thr;
- 10 (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites, or more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.*,  
20 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, *Gene*, 34:315 (1985)], restriction selection mutagenesis [Wells *et al.*, *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO1031-encoding variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively  
25 small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant. Alanine is also typically preferred because it is the most common amino acid.  
30 Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*,

(W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

### C. Modifications of PRO1031 or PRO1122

5 Covalent modifications of PRO1031 or PRO1122 polypeptides are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO1031 or PRO1122 polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of a PRO1031 or PRO1122 polypeptide. Derivatization with bifunctional agents is useful, for  
10 instance, for crosslinking PRO1031 or PRO1122 to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO1031 or PRO1122 antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazo-acetyl)-2-phenylethane, glutaraldehyde, N-hydroxy-succinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as  
15 3,3'-dithiobis-(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)-dithio]propionimide.

Other modifications include deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  
20  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO1031 or PRO1122 polypeptide included within the scope of this invention comprises altering the native glycosylation pattern  
25 of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO1031 or PRO1122 polypeptide, and/or adding one or more glycosylation sites that are not present in the native sequence PRO1031 or PRO1122 polypeptide. Additionally, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the  
30 nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to PRO1031 or PRO1122 polypeptides may be accomplished by altering the amino acid sequence thereof. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the

native sequence PRO1031 or PRO1122 polypeptide (for O-linked glycosylation sites). The PRO1031 or PRO1122 amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO1031 or PRO1122 polypeptide at preselected bases such that codons are generated that will translate into the  
5 desired amino acids.

Another means of increasing the number of carbohydrate moieties on the PRO1031 or PRO1122 polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

10 Removal of carbohydrate moieties present on the PRO1031 or PRO1122 polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.*, 118:131  
15 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification of PRO1031 or PRO1122 comprises linking the PRO1031 or PRO1122 polypeptide, respectively, to one of a variety of nonproteinaceous  
20 polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

PRO1031 or PRO1122 polypeptides of the present invention may also be modified in a way to form chimeric molecules comprising a PRO1031 or PRO1122 polypeptide, respectively, fused to another, heterologous polypeptide or amino acid sequence. In one  
25 embodiment, such a chimeric molecule comprises a fusion of a PRO1031 or PRO1122 polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO1031 or PRO1122 polypeptide. The presence of such epitope-tagged forms of a  
30 PRO1031 or PRO1122 polypeptide can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO1031 or PRO1122 polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of a PRO1031 or PRO1122 polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule, such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble transmembrane domain deleted or inactivated) form of a PRO1031 or PRO1122 polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also U.S. Patent 5,428,130, issued June 27, 1995.

In yet a further embodiment, the PRO1031 or PRO1122 polypeptides of the present invention may also be modified in a way to form a chimeric molecule comprising a PRO1031 or PRO1122 polypeptide fused to a leucine zipper. Various leucine zipper polypeptides have been described in the art. See, e.g., Landschulz *et al.*, *Science* 240:1759 (1988); WO 94/10308; Hoppe *et al.*, *FEBS Letters* 344:1991 (1994); Maniatis *et al.*, *Nature* 341:24 (1989). It is believed that use of a leucine zipper fused to a PRO1031 or PRO1122 polypeptide may be desirable to assist in dimerizing or trimerizing soluble PRO1031 or PRO1122 polypeptide in solution. Those skilled in the art will appreciate that the leucine zipper may be fused at either the N- or C-terminal end of the PRO1031 or PRO1122 molecule.



D. Preparation of PRO1031 or PRO1122

The description below relates primarily to production of PRO1031 or PRO1122 by culturing cells transformed or transfected with a vector containing PRO1031 or PRO1122 polypeptide encoding nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO1031 or PRO1122 polypeptides. For instance, the PRO1031 or PRO1122 sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, e.g., Stewart *et al.*, *Solid-Phase Peptide Synthesis*, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.*, 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of PRO1031 or PRO1122 polypeptides may be chemically synthesized separately and combined using chemical or enzymatic methods to produce a full-length PRO1031 or PRO1122 polypeptide.

15

1. Isolation of DNA Encoding PRO1031

DNA encoding a PRO1031 or PRO1122 polypeptide may be obtained from a cDNA library prepared from tissue believed to possess the PRO1031 or PRO1122 mRNA and to express it at a detectable level. Accordingly, human PRO1031- or PRO1122-encoding DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The PRO1031- or PRO1122-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated synthetic procedures, oligonucleotide synthesis).

Libraries can be screened with probes (such as antibodies to a PRO1031 or PRO1122 polypeptide or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO1031 is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

30

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled

such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like  $^{32}\text{P}$ -labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

5 Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein (e.g., through sequence  
10 alignment using computer software programs such as ALIGN, DNASTAR, BLAST, BLAST-2, INHERIT and ALIGN-2 which employ various algorithms to measure homology).

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in  
15 Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

## 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO1031 or PRO1122 polypeptide production and cultured in conventional  
20 nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: A Practical  
25 Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example,  $\text{CaPO}_4$  and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used  
30 for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of

mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*, 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear  
 5 microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al.*, *Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the nucleic acid (*e.g.*, DNA) in the  
 10 vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic  
 15 host cells include Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD266,710, published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than  
 20 limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete  
 25 genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA, ptr3 phoA E15 (argF-lac)169 degP ompT kan<sup>r</sup>*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, in vivo  
 30 methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO1031- or PRO1122-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nature, *Nature* 290: 140 [1981]; EP

139,383 published 2 May 1995); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; *Fleer et al.*, *Bio/Technology*, 9: 968-975 (1991) such as e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, *J. Bacteriol.* 737 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906); Van den Berg *et al.*, *Bio/Technology* 8: 135 (1990)); *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); Sreekrishna *et al.*, *J. basic Microbiol.* 28: 265-278 [1988]); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA* 76: 5359-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 10 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Balance *et al.*, *Biochem. Biophys. Res. Commun.* 112: 284-289 [1983]; Tilburn *et al.*, *Gene* 26: 205-221 [1983]; Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, *EMBO J.* 4: 475-479 [1985]). Methylotropic yeasts are selected from the genera 15 consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeast may be found in C. Antony, *The Biochemistry of Methylotrophs* 269 (1982).

Suitable host cells for the expression of glycosylated PRO1031 or PRO1122 are derived from multicellular organisms. Examples of invertebrate cells include insect cells 20 such as *Drosophila* S2 and *Spodoptera Sf9*, *Spodoptera high5* as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.*, 36:59 (1977)); Chinese hamster 25 ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding the desired PRO1031 or PRO1122 polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO1031 or PRO1122 polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the PRO1031- or PRO1122-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the PRO1031-or PRO1122-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the PRO1031- or PRO1122-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the PRO1031 or PRO1122 polypeptide.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

PRO1031 or PRO1122 transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

Transcription of a DNA encoding a PRO1031 or PRO1122 polypeptide by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO1031 or PRO1122 coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO1031 or PRO1122.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO1031 or PRO1122 polypeptides in recombinant vertebrate cell culture are described in

Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

5 Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize  
10 specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as  
15 immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO1031 or PRO1122 polypeptide or against a synthetic  
20 peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO1031- or PRO1122-encoding DNA and encoding a specific antibody epitope.

#### 5. Purification of Polypeptide

Forms of PRO1031 or PRO1122 may be recovered from culture medium or from host  
25 cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO1031 or PRO1122 polypeptides can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

30 It may be desired to purify PRO1031 or PRO1122 from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-



75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO1031 or PRO1122 polypeptide. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular PRO1031 or PRO1122 polypeptide produced.

#### E. Uses for PRO1031

10 Nucleotide sequences (or their complement) encoding PRO1031 or PRO1122 polypeptides have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. PRO1031- or PRO1122-encoding nucleic acid will also be useful for the preparation of PRO1031 or PRO1122 polypeptides by the recombinant techniques described  
15 herein.

The full-length DNA59294-1381 nucleotide sequence (SEQ ID NO:2), full-length DNA62377-1381-1 nucleotide sequence (SEQ ID NO:4) or the full-length native sequence PRO1031 or PRO1122 nucleotide-encoding sequence, or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length PRO1031 or PRO1122 gene  
20 or to isolate still other genes (for instance, those encoding naturally-occurring variants of PRO1031, PRO1122 or the same from other species) which have a desired sequence identity to the PRO1031 or PRO1122 nucleotide sequence disclosed in Figure 2 (SEQ ID NO:2) or Figure 4 (SEQ ID NO:4), respectively. Optionally, the length of the probes will be about 20 to about 50 bases. The hybridization probes may be derived from the DNA59294-1381 or  
25 DNA62377-1381-1 nucleotide sequence of SEQ ID NO:2 or SEQ ID NO:4, respectively, as shown in Figure 2 or Figure 4, respectively, or from genomic sequences including promoters, enhancer elements and introns of native sequence PRO1031- or PRO1122-encoding DNA. By way of example, a screening method will comprise isolating the coding region of the PRO1031 or PRO1122 gene using the known DNA sequence to synthesize a selected probe  
30 of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as <sup>32</sup>P or <sup>35</sup>S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the PRO1031 or PRO1122 gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine which

members of such libraries the probe hybridizes to. Hybridization techniques are described in further detail in the Examples below.

Any EST sequence (or fragment thereof) disclosed in the present application may similarly be employed as probes, using the methods disclosed herein.

5 Other useful fragments of the PRO1031 or PRO1122 nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target PRO1031 or PRO1122 mRNA (sense) of PRO1031 or PRO1122 DNA (anti-sense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of PRO1031 or PRO1122 DNA.  
10 Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659 (1988) and van der Krol *et al.*, *BioTechniques* 6: 958 (1988).

Binding of antisense or sense oligonucleotides to target nucleic acid sequences results  
15 in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense oligonucleotides thus may be used to block expression of PRO1031 or PRO1122 proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester  
20 backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable in vivo (*i.e.*, capable of resisting enzymatic degradation) but retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides  
25 which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such poly-L-lysine. Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target  
30 nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example, CaPO<sub>4</sub>-mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted

into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated CDT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related PRO1031 or PRO1122 sequences.

Nucleotide sequences encoding a PRO1031 or PRO1122 polypeptide can also be used to construct hybridization probes for mapping the gene which encodes that PRO1031 or PRO1122 polypeptide and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

When the coding sequences for PRO1031 or PRO1122 encode a protein which binds to another protein (example, where the PRO1031 or PRO1122 polypeptide, respectively, functions as a receptor), the PRO1031 or PRO1122 polypeptide, respectively, can be used in assays to identify the other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor PRO1031 or PRO1122 polypeptide can be used to isolate correlative ligand(s). Screening assays can be designed to find lead compounds that mimic the biological activity of a native PRO1031 or PRO1122 or

a receptor for PRO1031 or PRO1122, respectively. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

Nucleic acids which encode PRO1031 or PRO1122 polypeptide or any of its modified forms can also be used to generate either transgenic animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. A transgenic animal (*e.g.*, a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, *e.g.*, an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding PRO1031 or PRO1122 polypeptide can be used to clone genomic DNA encoding PRO1031 or PRO1122 in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding PRO1031 or PRO1122. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for PRO1031 or PRO1122 transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding PRO1031 or PRO1122 introduced into the germ line of the animal at an embryonic stage can be used to examine the effect of increased expression of DNA encoding PRO1031 or PRO1122. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, non-human homologues of PRO1031 or PRO1122 can be used to construct a PRO1031 or PRO1122, respectively, "knock out" animal which has a defective or altered gene encoding PRO1031 or PRO1122, respectively, as a result of homologous recombination between the endogenous gene encoding PRO1031 or PRO1122, respectively, and altered genomic DNA encoding PRO1031 or PRO1122, respectively, introduced into an embryonic cell of the animal. For example, cDNA encoding PRO1031 or PRO1122,

respectively, can be used to clone genomic DNA encoding PRO1031 or PRO1122, respectively, in accordance with established techniques. A portion of the genomic DNA encoding PRO1031 or PRO1122, respectively, can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration.

5 Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see *e.g.*, Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see *e.g.*, Li *et al.*, *Cell*, 69:915 (1992)].

10 The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse or rat) to form aggregation chimeras [see *e.g.*, Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the

15 homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the PRO1031 or PRO1122 polypeptide.

20 Nucleic acid encoding the PRO1031 or PRO1122 polypeptides may also be used in gene therapy. In gene therapy applications, gene are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents,

25 which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane.

30 Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA* 83: 4143-4146 [1986]]. The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into

cultured cell in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, *etc.* The currently preferred in vivo gene transfer techniques include  
5 transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, *Trends in Biotechnology* 11: 205-210 [1993]). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cells, *etc.* Where liposomes are employed, proteins which bind to a cell  
10 surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.*, capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, protein that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example by Wu *et al.*, *J. Biol. Chem.* 262: 4429-4432  
15 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 3410-3414 (1990). For a review of gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256: 808-813 (1992).

The PRO1031 or PRO1122 polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes.

20 The nucleic acid molecule encoding the PRO1031 or PRO1122 polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data are presently available. Each PRO1031 or PRO1122 nucleic acid molecule of the present invention can be used as a  
25 chromosome marker.

The PRO1031 or PRO1122 polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the PRO1031 or PRO1122 polypeptides of the present invention may be differentially expressed in one tissue as compared to another. PRO1031 or PRO1122 nucleic acid molecules will find use for  
30 generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

PRO1031 or PRO1122 polypeptides of the present invention which possess biological activity related to that of IL-17 may be employed both *in vivo* for therapeutic purposes and *in vitro*. Those of ordinary skill in the art will well know how to employ the PRO1031 or PRO1122 polypeptides of the present invention for such purposes.

PRO1031 or PRO1122 can be used in assays with the polypeptides to which they have identity with to determine the relative activities. The results can be applied accordingly.

An alignment of the predicted amino acid sequence of IL-17B (e.g., UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) with the known sequence of IL-17 (SEQ ID NO:11), show that this is a family of related sequences with a 26-28% amino acid identity between the three members (Figure 7). All three polypeptides contain a hydrophobic sequence at the N-terminus that is expected to function as a secretion signal sequence of 18-20 amino acids, giving a predicted size range for the members of this family 155 to 197 amino acids (mature MW 17 to 20 kDa). The alignment of Figure 7 shows several conserved amino acids, including a tryptophan residue and 5 cysteines in the C-terminal half of the proteins.

The PRO1031 or PRO1122 encoding nucleic acid or fragments thereof can also be used for chromosomal localizations. For example, the chromosome localization of IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) was determined using Taqman primers and probes designed in the 3'-untranslated regions of the IL-17B and IL-17C, was performed by PCR with Stanford Radiation Hybrid Panel G3 panel. IL-17B (UNQ516)(SEQ ID NO:1) mapped to human chromosome 5q32-34, whereas IL-17C (UNQ561)(SEQ ID NO:3) was localized to chromosome 16q24. Human IL-17 itself is found on chromosome 2q31. Rouvier *et al*, *M. Immunol.* 150: 5445 (1993).

The isolation and characterization of the two new relatives of IL-17, Applicants have established and expanded the potential role of this family of cytokines may play in proinflammatory immune and other responses. The three members of the family, IL-17 (SEQ ID NO:11), IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3), are modestly related in primary structure with about 27% overall amino acid identity including 5 conserved cysteine residues (Figure 7). The three family members share a number of features - they are 150-200 amino acid residues in length, they are secreted from cells via a hydrophobic secretion signal sequence, and they are expressed as disulfide-linked homodimers that in some cases appear to be glycosylated.

While members of the same gene family based on amino acid sequence similarity, the three proteins are expressed in different tissues and are dispersed in the genome. IL-17 expression (SEQ ID NO:11) has been reported only in activated T-cells, *Fossiez et al.*, *J. Exp. Med.* 183: 2593 (1996), Yao *et al.*, *J. Immunol.* 155: 5483 (1995)[Yao-3], while it is demonstrated herein that IL-17B (DNA59294)(SEQ ID NO:2) is expressed in normal human

adult pancreas, small intestine, and stomach (Figure 8). The expression pattern of IL-17C (DNA62377)(SEQ ID NO:4), however, is much more restricted, as confirmed expression in other tissues has not yet been discovered.

The characterizations described herein demonstrate that the biological activity of IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) are considerably different from the established activities for IL-17 (SEQ ID NO:11). IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) each fail to induce IL-6 production in human foreskin fibroblasts (Example 10)(Figure 9A). This is in contrast to the marked induction known for IL-17 (SEQ ID NO:11). Yao *et al.*, *Immunity* 3:811 (1995)[Yao-1], Yao *et al.*, *J. Immunol.* 155:5483 (1995)[Yao-3]. Conversely, IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3), each induce the release of TNF- $\alpha$  from the monocytic cell line, THP1, while IL-17 has only a very small effect (Figure 9B). The stimulated release of TNF- $\alpha$  in THP1 cells by IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3) is time and concentration dependent, (Example 10)(Figure 10), with IL-17B (SEQ ID NO:1) being about 10-fold more potent than IL-17C (SEQ ID NO:3) [ $EC_{50}$  = 2.4 nM for IL-17B vs. 25 nM for IL-17C].

The different biological effects of IL-17 (SEQ ID NO:11) as compared to IL-17B or C (SEQ ID NO:s 1 & 3), suggests that they may function via a different cell surface receptor (or some differing receptor components) than the known IL-17 receptor. Yao *et al.*, *Cytokine* 9:794 (1997) [Yao-3]. In an effort to examine the question of receptor specificity directly, Applicants have demonstrated that both IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3) fail to bind to the IL-17 receptor ECD (SEQ ID NO:16)(Figure 11A), and also fail to compete for the binding of IL-17 (SEQ ID NO:11) to its receptor ECD (SEQ ID NO:16)(Figure 11B). IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3) do bind to the surface of THP1 cells, where they have activity (Figure 12). The interaction is specific at least to the extent that a control Fc fusion protein fails to bind to these cells. The results suggest that there could be a set of receptors that bind and transduce the signal from the family of IL-17 cytokines, a receptor/ligand model that has been found for many cytokine and growth factor families.

The novel cytokines disclosed herein, PRO1031 (*e.g.*, 516) and PRO1122 (*e.g.*, UNQ561), differ from IL-17 (SEQ ID NO:11) in their patterns of expression and biological activities. The differential expression coupled with the lack of interaction with the known IL-17 receptor suggests and expanded role for the IL-17 family in the proinflammatory immune response.



## F. Anti-PRO1031 and anti-PRO1122 Antibodies

The present invention further provides anti-PRO1031 and anti-PRO1122 polypeptide antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

### 5 1. Polyclonal Antibodies

The anti-PRO1031 or anti-PRO1122 antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the  
10 immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO1031 or PRO1122 polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum  
15 albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

### 20 2. Monoclonal Antibodies

The anti-PRO1031 or anti-PRO1122 antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent  
25 to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include the PRO1031 or PRO1122 polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used  
30 if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly

myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine  
5 phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a  
10 medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984);  
15 Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against a PRO1031 or PRO1122 polypeptide. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells  
20 is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.*, 107:220 (1980).

25 After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

30 The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81, 6851-6855 (1984)] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### 3. Humanized Antibodies

The anti-PRO1031 or anti-PRO1122 antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-

human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or complete inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects,

including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

#### 4. Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO 81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U. S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, glycosidase, glucose oxidase, human lysosyme, human glucuronidase, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases (*e.g.*, carboxypeptidase G2 and carboxypeptidase A) and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase useful for converting drugs derivatized with  $\beta$ -lactams into free drugs; and penicillin amidases, such as penicillin Vamidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes" can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-PRO1031 or anti-PRO1122 antibodies by techniques well known in the art such as the use of the heterobifunctional cross-linking agents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of the antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.* Neuberger *et al.*, *Nature* 312: 604-608 (1984)).

#### 5. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a PRO1031 polypeptide, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, *Nature*, 305:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small

amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g.  $F(ab')_2$  bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using chemical linkage. Brennan *et al.*, *Science* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate  $F(ab')_2$  fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol- by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody  $F(ab')_2$  molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various technique for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers, Kostelny *et al.*, *J. Immunol.* 148(5): 1547-1553 (1992), wherein the leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-

6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain ( $V_H$ ) connected to a light-chain variable domain ( $V_L$ ) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the  $V_H$  and  $V_L$  domains of one fragment are forced to pair with the complementary  $V_L$  and  $V_H$  domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152: 5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147: 60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given "Pro" protein herein. Alternatively, an anti-"PRO" protein arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular "PRO" protein. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular "PRO" polypeptide. These antibodies possess a "PRO"-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the "PRO" polypeptide and further binds tissue factor (TF).

#### 6. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.



## 7. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design* 3: 219-230 (1989).

## 8. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof; or a small molecule toxin), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active protein toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin; cholera toxin, botulinus toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, saporin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. Small molecule toxins include, for example, calicheamicins, maytansinoids, palytoxin and CC1065. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include  $^{212}\text{Bi}$ ,  $^{131}\text{I}$ ,  $^{131}\text{In}$ ,  $^{90}\text{Y}$  and  $^{186}\text{Re}$ .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (*p*-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(*p*-diazoniumbenzoyl)-ethylenediamine), diisocyanates

(such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for  
 5 conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated  
 10 to a cytotoxic agent (*e.g.* a radionucleotide).

#### 9. Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al.*, *Proc.*  
 15 *Natl. Acad. Sci. USA*, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of  
 20 defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.* 81(19): 1484 (1989).

#### 25 10. Pharmaceutical Compositions of Antibodies

Antibodies specifically binding a PRO1031 or PRO1122 polypeptide identified herein, as well as other molecules identified by the screening assays disclosed hereinbefore, can be administered for the treatment of various disorders in the form of pharmaceutical compositions.

30 If a PRO1031 or PRO1122 polypeptide is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region

sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco et al., *Proc. Natl. Acad. Sci. USA* 90: 7889-7893 (1993).

5           The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokines, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitable  
10 present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or felatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes,  
15 albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, *supra*.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

20           Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat.  
25 No. 3,773,919), copolymers of L-glutamic acid  $\gamma$ -ethyl-L-glutamate, non-degradable ethylene-vinylacetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days,  
30 certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the

mechanisms involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thiosulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

G. Uses for anti-PRO1031 and anti-PRO1122 Antibodies

The anti-PRO1031 and anti-PRO1122 antibodies of the present invention have various utilities. For example, anti-PRO1031 or anti-PRO1122 antibodies may be used in diagnostic assays for PRO1031 or PRO1122 polypeptides, e.g., detecting expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogeneous phases [Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , or  $^{125}\text{I}$ , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature*, 144:945 (1962); David *et al.*, *Biochemistry*, 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth.*, 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.*, 30:407 (1982).

Anti-PRO1031 or anti-PRO1122 antibodies also are useful for the affinity purification of PRO1031 or PRO1122 polypeptides, respectively, from recombinant cell culture or natural sources. In this process, the antibodies against a PRO1031 or PRO1122 polypeptide are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the PRO1031 or PRO1122 polypeptide to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO1031 or PRO1122 polypeptide, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the PRO1031 or PRO1122 polypeptide from the antibody.

#### H. PRO1031, PRO1122 and IL-17 Antagonists/Agonists

This invention encompasses methods of screening compounds to identity those that mimic the PRO1031, PRO1122 or IL017 polypeptide (agonists) or prevent the effect of the PRO1031, PRO1122 or IL-17 polypeptide (antagonists). Screening assays for antagonist  
5 drug candidates are designed to identity compounds that bind or complex with the PRO1031, PRO1122, IL-17 polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates.

10 The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art. For example, to screen for antagonists and/or agonists of PRO1031, PRO1122, IL-17 signaling, the assay mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, PRO1031, PRO1122 or IL-17 induces  
15 TNF- $\alpha$  release from THP-1 cells with a reference activity. Alternatively, the tested activity can be the release of nitric oxide (NO) and proteoglycans from IL17 and/or IL-1 $\alpha$  treated articular cartilage.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the PRO1031, PRO1122 or  
20 IL-17 polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the PRO1031, PRO1122 or IL-17 polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the PRO1031, PRO1122 or IL-17  
25 polypeptide to be immobilized can be used to anchor it to solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and  
30 complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does

not carry a label, complexing can be detected, for example, by using a labeled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular PRO1031, PRO1132 or IL-17 polypeptide encoded by a gene identified herein, its interaction with that polypeptide can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, e.g., cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers (Fields and Song, *Nature* 340: 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 9578-9582 (1991) as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* 89: 5789-5791 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other functions as the transcription-activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptide are detected with chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER<sup>TM</sup>) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

Compounds that interfere with the interaction of a gene encoding a PRO1031, PRO1122 or IL-17 polypeptide identified herein and other intra- or extracellular components can be tested as follows: usually a reaction mixture is prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a candidate compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as a positive control.

The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described hereinabove. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

Antagonists may be detected by combining the PRO1031, PRO1122 or IL-17 polypeptide and a potential antagonist with membrane-bound PRO1031, PRO1122 or IL-17 polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The PRO1031, PRO1122 or IL-17 polypeptide can be labeled, such as by radioactivity, such that the number of PRO1031, PRO1122 or IL-17 polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan *et al.*, *Current Protocols in Immun.* 1(2): Ch. 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the PRO1031- or PRO1122 polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the PRO1031, PRO1122 or IL-17 polypeptide, respectively. Transfected cells that are grown on glass slides are exposed to labeled PRO1031, PRO1122 or IL-17 polypeptide. The PRO1031, PRO1122 or IL-17 polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

As an alternative approach for receptor identification, labeled PRO1031, PRO1122 or IL-17 polypeptide can be photoaffinity-linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE and exposed to X-ray film. The labeled complex containing the receptor can be excised, resolved into peptide fragments, and subjected to protein micro-sequencing. The amino acid sequence obtained from micro-sequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the gene encoding the putative receptor.

In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled PRO1031, PRO1122 or IL-17

polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be removed.

More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of immunoglobulin with PRO1031, PRO1122 or IL-17 polypeptide, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the PRO1031, PRO1122 or IL-17 polypeptide that recognizes the receptor but impart no effect, thereby competitively inhibiting the action of the PRO1031, PRO1122 or IL-17 polypeptide.

Another potential PRO1031, PRO1122 or IL-17 polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, e.g., an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing its translation into protein. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature PRO1031, PRO1122 or IL-17 polypeptides herein, is used to design an antisense RNA oligonucleotide sequence, which encodes the mature PRO1031, PRO1122 or IL-17 polypeptides herein, is used to design an antisense RNA oligonucleotide of about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6: 3073 (1979); Cooney *et al.*, *Science* 241: 456 (1988); Dervan *et al.*, *Science* 251: 1360 (1991)), thereby preventing transcription and the production of the PRO1031 or PRO1122 polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the PRO1031, PRO1122 or IL-17 polypeptide (antisense - Okano, *Neurochem.* 546: 560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression* (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the PRO1031, PRO1122, IL-17 polypeptide. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation-initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.



Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the PRO1031 or PRO1122 polypeptide, thereby blocking the normal biological activity of the PRO1031, PRO1122 or IL-17 polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonuclytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details, see e.g., Rossi, Current Biology 4: 469-471 (1994) and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCR publication No. WO 97/33551, *supra*.

#### I. Diagnostic Uses

Another use of the compounds of the invention (e.g., PRO1031- or PRO1122-variants and anti-PRO1031 or anti-PRO1122 antibodies) described herein is to help diagnose whether a disorder is driven, to some extent, by PRO1031 or PRO1122 modulated signaling.

A diagnostic assay to determine whether a particular disorder (e.g., degenerative cartilaginous disorder) is driven by PRO1031 or PRO1122 signaling, can be carried out using the following steps: (1) culturing test cells or tissues expressing PRO1031 or PRO1122; (2) administering a compound which can inhibit PRO1031 or PRO1122 modulated signaling; and (3) measuring the PRO1031 or PRO1122 mediated phenotypic effects in the test cells. The steps can be carried out using standard techniques in light of the present disclosure. For example, standard techniques can be used to isolate cells or tissues and culturing or *in vivo*.

Compounds of varying degree of selectivity are useful for diagnosing the role of PRO1031 or PRO1122. For example, compounds which PRO1031 or PRO1122 in addition to another form of adaptor molecule can be used as an initial test compound to determine if one of several adaptor molecules drive the disorder. The selective compounds can then be used to further eliminate the possible role of the other adaptor proteins in driving the disorder.

Test compounds should be more potent in inhibiting intracellular signaling activity than in exerting a cytotoxic effect (e.g., an  $IC_{50}/LD_{50}$  of greater than one). The  $IC_{50}$  and  $LD_{50}$  can be measured by standard techniques, such as an MTT assay, or by measuring the amount of LDH released. The degree of  $IC_{50}/LD_{50}$  of a compound should be taken into account in evaluating the diagnostic assay. Generally, the larger the ratio the more relative the information. Appropriate controls take into account the possible cytotoxic effect of a compound of a compound, such as treating cells not associated with a cell proliferative disorder (e.g., control cells) with a test compound, can also be used as part of the diagnostic assay. The diagnostic methods of the invention involve the screening for agents that modulate the effects of PRO1031 or PRO1122 upon degenerative cartilagenous disorders. Exemplary detection techniques include radioactive labeling and immunoprecipitating (U.S.P. 5,385,915).

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by the disease-related genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g. fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art.

*In situ* detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

#### J. Pharmaceutical Compositions

The PRO1031 or PRO1122, antagonists or agonists thereof (e.g., antibodies), as well as other molecules identified by the screening assays disclosed hereinbefore, can be employed as therapeutic agents. Such therapeutic agents are formulated according to known methods to prepare pharmaceutically useful compositions, whereby the PRO1031 or PRO1122, antagonist or agonist thereof is combined in admixture with a pharmaceutically acceptable carrier.

In the case of PRO1031 or PRO1122 antagonist or agonist antibodies, if the protein encoded by the amplified gene is intracellular and whole antibodies are used as inhibitors,

internalizing antibodies are preferred. However, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, *e.g.* Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 7889-7893 [1993]).

Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and *m*-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in

macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

5       Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, and intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or  
10       microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$ -ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON  
15       DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- (rhIFN-), interleukin-2, and MN rpg 120. Johnson et al., Nat. Med. 2: 795-799 (1996); Yasuda et al., Biomed. Ther. 27: 1221-1223 (1993); Hora et al.,  
20       Bio/Technology 8: 755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds., (Penum Press: New York, 1995), pp. 439-462; WO 97/03692; WO 96/40072; WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins may be developed using poly  
25       lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer", in  
30       *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker; New York, 1990), M. Chasin and R. Langer (Eds.) pp. 1-41.

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time

periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

#### K. Methods of Treatment

It is contemplated that the compounds of the present invention may be used to treat various conditions, including those characterized by overexpression and/or activation of the disease-associated genes identified herein. Exemplary conditions or disorders to be treated with such antibodies and other compounds, including, but not limited to, small organic and inorganic molecules, peptides, antisense molecules, *etc.* include inflammatory and immunologic disorders, especially those characterized by cartilage matrix breakdown such as arthritis, (*e.g.*, osteoarthritis, psoriatic arthritis, rheumatoid arthritis) or other degenerative inflammatory diseases.

The active agents of the present invention, *e.g.* antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebral, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, intraocular, intralesional, oral, topical, inhalation or through sustained release.

Other therapeutic regimens may be combined with the administration of the PRO1031, PRO1122, antagonists or agonists, anti-cancer agents, *e.g.* antibodies of the instant invention.

For the prevention or treatment of disease, the appropriate dosage of an active agent, (*e.g.* an antibody) will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of

the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in  
 5 Toxicokinetics," In Toxicokinetics and New Drug Development, Yacobi et al., Eds. Pergamon Press, New York 1989, pp.42-46.

When *in vivo* administration of a PRO1031 or PRO1122 polypeptide or agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg upto 100 mg/kg of mammal body weight or more per day, preferably about 1µg/kg/day up to 100  
 10 mg/kg of mammal body weight or more pre day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760, 5,206,344 or 5,255,212. It is within the scope of the invention that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may  
 15 necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily  
 20 monitored by conventional techniques and assays.

#### L. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the diagnosis or treatment of the disorders described above is provided.  
 25 The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a  
 30 hypodermic injection needle). The active agent in the composition is typically a PRO1031, PRO1122 polypeptide, antagonist, or agonist thereof. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a

pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

5 \*\*\*\*\*

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

10

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the  
15 American Type Culture Collection, Rockville, Maryland.

#### EXAMPLE 1: Isolation of cDNA Clones Encoding Human PRO1031

The extracellular domain (ECD) sequences (including the secretion signal, if any) of from about 950 known secreted proteins from the Swiss-Prot public protein database were  
20 used to search expressed sequence tag (EST) databases. The EST databases included public EST databases (e.g., GenBank, Merck/Wash U.) and a proprietary EST DNA database (LIFESEQ<sup>®</sup>, Incyte Pharmaceuticals, Palo Alto, CA). The search was performed using the computer program BLAST or BLAST2 (Altshul *et al.*, *Methods in Enzymology* 266:460-480 (1996)) as a comparison of the ECD protein sequences to a 6 frame translation of the EST  
25 sequence. Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington).

An initial virtual sequence fragment (consensus assembly) was assembled relative to  
30 other EST sequences using phrap. The initial consensus DNA sequence was extended using repeated cycles of BLAST and phrap to extend the consensus sequence as far as possible using the sources of EST sequences discussed above. The results of this assembly is shown in Figure 5 (SEQ ID NO:5), also referred to as DNA47332.

One sequence comprising the consensus assembly, W74558 (clone 344649)(SEQ ID NO:6) was further examined. The sequence was obtained from the IMAGE consortium and analyzed. Lennon *et al.*, *Genomics* 33: 151 (1996). DNA sequencing gave the full-length DNA sequence for PRO1031 [herein designated as DNA59294-1381] (SEQ ID NO:2) and the derived PRO1031 protein sequence (UNQ516)(SEQ ID NO: 1).

The entire nucleotide sequence of DNA59294-1381 is shown in Figure 2 (SEQ ID NO:2). Clone DNA59294-1381 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 42-44 and ending at the stop codon at nucleotide positions 582-584 (Figure 2)(SEQ ID NO:2). The predicted polypeptide precursor is 180 amino acids long (Figure 1)(SEQ ID NO:1). The full-length PRO1031 (UNQ516) protein shown in Figure 2 (SEQ ID NO:1) has an estimated molecular weight of about 20437 and a pI of about 9.58. Clone DNA59294-1381 (SEQ ID NO:2) has been deposited with the ATCC, and have been assigned deposit number 209866. In the event of any sequencing irregularities or errors with the sequences provided herein, it is understood that the deposited clone contains the correct sequence for DNA59624 (SEQ ID NO:2). Furthermore, the sequences provided herein are the result of known sequencing techniques.

Analysis of the amino acid sequence of the full-length PRO1031 polypeptide (UNQ516)(SEQ ID NO:1) suggests that it is a novel cytokine.

Further analysis of the amino acid sequence of SEQ ID NO:2 reveals that the putative signal peptide is at about amino acids 1-20 of SEQ ID NO:2. An N-glycosylation site is at about amino acids 75-78 of SEQ ID NO:2. A region having sequence identity with IL-17 is at about amino acids 96-180. The corresponding nucleotides can be routinely determined given the sequences provided herein.

## EXAMPLE 2: Isolation of cDNA clones Encoding Human PRO1122

An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST was identified. The EST was Incyte 1347523 (SEQ ID NO:7) also called DNA49665. Based on DNA49665 (SEQ ID NO:7), oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolated a clone of the full-length coding sequence for the PRO1122. [e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989); Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].



Forward and reverse PCR primers generally range from 20 to 30 nucleotides and are often designed to give a PCR product of about 100-1000 bp in length. The probes sequences are typically 40-55 bp in length. In some cases, additional oligonucleotides are synthesized when the consensus sequence is greater than about 1-1.5 kpb. In order to screen several  
 5 libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausuble *et al.*, *Current Protocols in Molecular Biology*, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

PCR primers (forward, reverse and hybridization) were synthesized:

10 forward PCR primer: 5'-ATCCACAGAAGCTGGCCTTCGCCG-3' (SEQ ID NO:8)

reverse PCR primer: 5'-GGGACGTGGATGAACTCGGTGTGG-3' (SEQ ID NO:9)

hybridization probe:

5'-TATCCACAGAAGCTGGCCTTCGCCGAGTGCCTGTGCAGAG-3' (SEQ ID NO:10).

In order to screen several libraries for a source of a full-length clone, DNA from the  
 15 libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO1122 gene using the probe oligonucleotide and one of the PCR primers.

RNA for construction of the cDNA libraries was isolated from human fetal kidney tissue. The cDNA libraries used to isolate the cDNA clones were constructed using standard  
 20 methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes *et al.*,  
 25 *Science* 235: 1278-1280 (1991)) in the unique XhoI and NotI sites.

DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for PRO1122 [herein designated as DNA62377-1381-1](SEQ ID NO:4) and the derived protein PRO1122 sequence (UNQ561)(SEQ ID NO:3).

The entire nucleotide sequence of DNA62377-1381-1 (SEQ ID NO:4) is shown in  
 30 Figures 4A-4B (SEQ ID NO:4). Clone DNA62377-1381-1 (SEQ ID NO:4) contains a single open reading frame with an apparent translational initiation site at nucleotide positions 50-52 and ending at the stop codon at nucleotide positions 641-643 of SEQ ID NO:4 (Figures 4A-4B). The predicted polypeptide precursor is 197 amino acids long (Figure 3)(SEQ ID NO:3). The full-length PRO1122 protein shown in Figure 3 (UNQ561)(SEQ ID NO:3) has an

estimated molecular weight of about 21765 daltons and a pI of about 8.53. Clone DNA62377-1381-1 has been deposited with the ATCC on December 22, 1998 and has been assigned deposit number 203552. It is understood that in the event of a sequencing irregularity or error in the sequences provided herein, the correct sequence is the sequence deposited. Furthermore, all sequences provided herein are the result of known sequencing techniques.

Analysis of the amino acid sequence of the isolated full-length PRO1122 (UNQ561) suggests that it possesses similarity with IL-17, thereby indicating that PRO1122 (UNQ561) may be a novel cytokine. Figure 3 (SEQ ID NO:3) also shows the approximate locations of the signal peptide, leucine zipper pattern, and a region having sequence identity with IL-17. The corresponding nucleotides can be routinely determined, e.g., by reference to Figures 4A-4B.

#### EXAMPLE 3: Use of PRO1031- or PRO1122-encoding DNA as a hybridization probe

The following method describes use of a nucleotide sequence encoding PRO1031 as a hybridization probe.

DNA comprising the coding sequence of full-length PRO1031 (as shown in Figure 2, SEQ ID NO:2), PRO1122 (as shown in Figure 4, SEQ ID NO:4) or a fragment thereof is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO1031 or PRO1122 in human tissue cDNA libraries or human tissue genomic libraries).

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO1031 or PRO1122 polypeptide-derived probe to the filters is performed in a solution of 50% formamide, 5 x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2 x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing of the filters is performed in an aqueous solution of 0.1 x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO1031 or PRO1122 polypeptide can then be identified using standard techniques known in the art.

#### EXAMPLE 4: Expression of PRO1031 or PRO1122 Polypeptides in *E. coli*

This example illustrates the preparation of unglycosylated forms of PRO1031 or PRO1122 polypeptides by recombinant expression in *E. coli*.

The DNA sequence encoding the full-length PRO1031, PRO1122 or a fragment or variant thereof is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO1031 or PRO1122 coding region, lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO1031 or PRO1122 polypeptide can then be purified using a metal chelating column under conditions that allow tight binding of the polypeptide.

#### EXAMPLE 5: Expression of PRO1031 or PRO1122 Polypeptides in Mammalian Cells

This example illustrates preparation of glycosylated forms of PRO1031 or PRO1122 polypeptides by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO1031- or PRO1122-encoding DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO1031- or PRO1122-encoding DNA using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called pRK5-PRO1031 or pRK5-PRO1122, respectively.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-PRO1031 or pRK5-PRO1122 DNA is mixed with about 1 µg  
5 DNA encoding the VA RNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is  
10 aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine  
15 and 200 µCi/ml <sup>35</sup>S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO1031 or PRO1122 polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

20 In an alternative technique, PRO1031- or PRO1122-encoding DNA may be introduced into 293 cells transiently using the dextran sulfate method described by Sompayrac *et al.*, *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-PRO1031 or pRK5-PRO1122 DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with  
25 PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO1031 or  
30 PRO1122 polypeptide can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO1031 or PRO1122 polypeptide can be expressed in CHO cells. The pRK5-PRO1031 or pRK5-PRO1122 vector can be transfected into CHO cells using known reagents such as CaPO<sub>4</sub> or DEAE-dextran. As described above, the cell cultures

can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as  $^{35}\text{S}$ -methionine. After determining the presence of PRO1031 or PRO1122 polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO1031 or PRO1122 polypeptide can then be concentrated and purified by any selected method.

Epitope-tagged PRO1031 or PRO1122 polypeptide may also be expressed in host CHO cells. The PRO1031- or PRO1122-encoding DNA may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO1031- or PRO1122-encoding DNA insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO1031 or PRO1122 polypeptide can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

#### EXAMPLE 6: Expression of a PRO1031 Polypeptide in Yeast

The following method describes recombinant expression of PRO1031 or PRO1122 polypeptides in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO1031 or PRO1122 polypeptide from the ADH2/GAPDH promoter. DNA encoding the PRO1031 or PRO1122 polypeptide of interest, a selected signal peptide and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of the PRO1031 or PRO1122 polypeptide. For secretion, DNA encoding the PRO1031 or PRO1122 polypeptide can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, the yeast alpha-factor secretory signal/leader sequence, and linker sequences (if needed) for expression of the PRO1031 or PRO1122 polypeptide.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant PRO1031 or PRO1122 polypeptide can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing the PRO1031 or PRO1122 polypeptide may further be purified using selected column chromatography resins.

EXAMPLE 6: Expression of PRO1031 or PRO1122 Polypeptides in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO1031 or PRO1122 polypeptides in Baculovirus-infected insect cells.

The PRO1031- or PRO1122-encoding DNA is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the PRO1031- or PRO1122-encoding DNA or the desired portion of the PRO1031- or PRO1122-encoding DNA (such as the sequence encoding the extracellular domain of a transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 to 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression is performed as described by O'Reilley *et al.*, *Baculovirus Expression vectors: A Laboratory Manual*, Oxford:Oxford University Press (1994).

Expressed poly-his tagged PRO1031 or PRO1122 polypeptide can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, *Nature*, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10%

Glycerol, pH 7.8) and filtered through a 0.45  $\mu$ m filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged PRO1031 polypeptide are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) PRO1031 polypeptide can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

#### EXAMPLE 7: Preparation of Antibodies that Bind PRO1031 or PRO1122 Polypeptides

This example illustrates the preparation of monoclonal antibodies which can specifically bind to PRO1031 or PRO1122 polypeptides.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO1031 or PRO1122 polypeptide, fusion proteins containing a PRO1031 or PRO1122 polypeptide, and cells expressing recombinant PRO1031 or PRO1122 polypeptide on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

Mice, such as Balb/c, are immunized with the PRO1031 or PRO1122 immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO1031 or anti-PRO1122 polypeptide antibodies.

After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO1031 or PRO1122 polypeptide. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

The hybridoma cells will be screened in an ELISA for reactivity against PRO1031 or PRO1122 polypeptide. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against a PRO1031 or PRO1122 polypeptide is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO1031 or anti-PRO1122 polypeptide monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

#### Example 8: RNA Expression

Multi-tissue blots containing poly A<sup>+</sup> RNA (2μg per lane) from various human tissues were purchased from Clontech (Palo Alto, CA). The entire coding regions of human IL-17B (UNQ516)(700 bp)(SEQ ID NO:17) and IL-17C (UNQ561) (1.1 kbp)(SEQ ID NO:18) were used as hybridization probes. DNA probes were labeled with [α-<sup>32</sup>P]-dCTP by random priming DNA labeling beads (Pharmacia Biotech). Hybridization was performed using Expresshyb (Clontech) containing the radiolabeled probes at 68°C for 1 hour. The blots were then washed with 2X SSC/0.05% SDS solution at room temperature for 40 minutes, followed by washes in 0.1X SSC/0.05% SDS solution at 55°C for 40 minutes with one change of fresh solution. The blots were exposed in a phosphorimager and the resulting image is reported herein as Figure 8.

For IL-17B (SEQ ID NO:1), an 800 bp mRNA transcript was found in pancreas, small intestine, and stomach of adult human tissues; a weaker band was detected in testis. (Figure



8). IL-17C (SEQ ID NO:2) expression was examined in the same set of adult human tissues, but no detectable signals were observed.

#### Example 9: Generating Fc/His fusion proteins

5 The coding sequences of IL17B (SEQ ID NO:17) and IL17C (SEQ ID NO:18) were amplified by PCR and subcloned into the EcoRI and SmaI sites of pBPH.His.c to generate a C-terminal GHHHHHHHH tag (SEQ ID NO:19) or the EcoRI and Stu sites of pBPH.IgG to generate a C-terminal fusion with the Fc region of human IgG1. Vectors pBPH.His.c and pBPH.IgG are derivatives of the baculovirus expression vector pVL1393 (Pharmingen). A  
10 control Fc or his-tagged protein was constructed in a similar way by C-terminally linking pancreatitis-associated protein (175 amino acid) to the Fc portion of the human IgG1 or a his8 tag.

The fusion proteins were expressed in H5 cells using the manufacturer's recommended procedure (Invitrogen). In brief, the DNA constructs were co-transfected with  
15 BaculoGold Baculovirus DNA (Pharmingen) in a 7:1 ratio into adherent Sf9 cells. Cells were incubated at 28°C for 4 days and the supernatant was harvested. The transfection supernatant was amplified and was subject to affinity purification by either protein A-sepharose beads (Pharmacia) for Fc fusion proteins or Ni-NTA agarose beads (QIAGEN) for His-tagged proteins.

20 To examine the protein expression, SDS-PAGE analysis was performed on the affinity purified recombinant proteins under non-reducing and reducing conditions, followed by silver staining.

#### Example 10: Induction of IL-6 and TNF- $\alpha$ release

25 Using the procedure outlined in Yao *et al.*, *J. Immunol.* **155**: 5483 (1995) (Yao-2) for IL-6 (SEQ ID NO:14) release, human foreskin fibroblast cells (ATCC CRL-2091) were cultured in MEM media (10% FBS) with the test cytokine. After incubation for 18 hours at 37°C and 5% CO<sub>2</sub>, conditioned media were assayed for IL-6 using an ELISA kit (R&D Systems). For TNF- $\alpha$  secretion, human leukemia monocytic THP-1 cells were cultured in  
30 RPMI media (10% FBS) with test cytokine. After incubation for 18 hour at 37°C and 5% CO<sub>2</sub>, conditioned media were quantitated for TNF- $\alpha$  (SEQ ID NO:20) using and ELISA assay kit (R&D Systems).

Human foreskin fibroblast cells (ATCC) were separately cultured in MEM media (10% FBS) in the presence of IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3). After incubation for 18 hours at 37°C and 5% CO<sub>2</sub>, conditioned media were assayed for IL-6 (SEQ ID NO:14) using an ELISA kit (R&D Systems). In contrast to the high level of IL-6 (SEQ ID NO:14) induced by IL-17 (SEQ ID NO:11), both IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3) failed to stimulate IL-6 (SEQ ID NO:14) secretion in fibroblast cells (Figure 9A).

Using the procedure outlined in Yao *et al.*, *Cytokine* 9: 794 (1997) [Yao-3], a human leukemic monocytic cell line, THP-1, was used to assay for the stimulation of TNF- $\alpha$  (SEQ ID NO:20) release by IL-17 (SEQ ID NO:11), UNQ516 (SEQ ID NO:1) and UNQ561 (SEQ ID NO:3) by culturing in RPMI media (10% FBS). After incubation for 18 hour at 37°C and 5% CO<sub>2</sub>, conditioned media were quantitated for TNF- $\alpha$  (SEQ ID NO:19) using and ELISA assay kit (R&D Systems). While IL-17 (SEQ ID NO:11) induced only a low level of TNF- $\alpha$  (SEQ ID NO:19) in THP-1 cells, both IL-17B and IL-17C (as Fc fusion proteins) stimulated TNF- $\alpha$  production in THP-1 cells (Figure 9B). A control Fc fusion protein had no effect.

In order to further characterize the stimulation of TNF- $\alpha$  release by IL-17B and IL-17C, the time course and concentration dependence of the response were assayed in THP-1 cells. Figure 10 illustrates that IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) stimulate the release of TNF- $\alpha$  (SEQ ID NO:19) in a time- and concentration-dependent manner. The EC<sub>50</sub> for IL-17B (UNQ516)(SEQ ID NO:1) stimulation is 2.4 nM, while for IL-17C (UNQ561)(SEQ ID NO:3), 25 nM.

While the IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) preparations used in these experiments contained undetectable level of endotoxin (less than 1 EU/ml), additional control experiments were performed to confirm that the TNF- $\alpha$  (SEQ ID NO:19) release from THP-1 cells was real and not artifactual. The IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) activities were unaffected by polymyxin B treatment and were abolished by heat treatment, further supporting the notion that the proteins themselves were responsible for the activities and not any contaminating endotoxin.

### Example 11: IL-17 Receptor Binding

#### *Cloning of the ECD of hIL-17 Receptor:*

In order to clone the ECD of the human IL-17 receptor, two oligonucleotide primers were designed at the 5' and 3' ends of IL-17R ECD (SEQ ID NO:15) based on the published sequence. *Yao et al., supra* (Yao-3). The two probes had the following sequences:

primer 1: 5'-CTG TAC CTC GAG GGT GCA GAG-3' (SEQ ID NO:20)

5 primer 2: 5'-CCC AAG CTT GGG TCA ATG ATG ATG ATG ATG ATG ATG CCA  
CAG GGG CAT GTA GTC C-3' (SEQ ID NO:21)

The above primers were used in PCR reactions to amplify the full-length cDNA from a human testis cDNA library with Pfu Turbo DNA polymerase (Promega). A C-terminal his tag was introduced by PCR through the addition of nucleotides encoding eight histidines to  
10 the 3' end primer. The PCR product was then subcloned into an expression plasmid vector pRK5B. Sequence analysis confirmed that the insert contains a DNA fragment encoding the extracellular domain (1-320 amino acids) of the published hIL-17 receptor. (SEQ ID NO:15).

#### 15 *Immunoprecipitation of the IL-17R ECD:*

The differential activity of IL-17 when compared to IL-17B (UNQ516)(SEQ ID NO:1) and IL-17C (UNQ561)(SEQ ID NO:3) suggested that they might bind and activate different cell surface receptors. In order to test whether IL-17B (UNQ516)(SEQ ID NO:1) or IL-17C (UNQ561)(SEQ ID NO:3) directly bind to the receptor, an expression plasmid  
20 containing the IL-17R (C-terminal his-tagged)(SEQ ID NO:22) was transfected into 293 cells using SuperFect transfection reagent (Quiagen). Metabolic labeling of 293 cells was performed 16 hours after transfection using 50  $\mu$ Ci/ml [ $^{35}$ S]-Cys/Met mixture for 6 hours. Conditioned medium was collected and concentrated (Centricon-10, Amicon). To examine the expression of the IL-17R ECD (SEQ ID NO:15), Ni-NTA beads (Quiagen) were used to  
25 affinity precipitate the his-tagged IL-17R ECD (SEQ ID NO:22) from the conditioned medium.

The conditioned medium was diluted in RIPA buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS in PBS) was incubated with IL-17 (SEQ ID NO:11) and the Fc fusion proteins overnight at 4°C. Protein A-agarose beads (Pierce) were added to precipitate  
30 the Fc fusion proteins. The precipitates were washed three times to precipitate the Fc fusion proteins. The precipitates were washed three times in RIPA buffer, denatured in SDS sample buffer, and electrophoresed on NuPAGE 4-12% Bis-Tris gels (Novex). For IL-17 (SEQ ID NO: 11) immunoprecipitation, anti-IL-17 antibody (R&D Systems) was added. In a

competitive binding experiment, immunoprecipitation of IL-17R ECD (SEQ ID NO:15) by IL-17 (SEQ ID NO:11) is performed in the presence of a 5-fold molar excess of IL-17B.his (SEQ ID NO:23, IL-17C.his (SEQ ID NO:24 and control his tagged protein.

The IL-17R ECD (SEQ ID NO:15) migrated as a 60 kDa band when purified via its histidine tag (Fig 11A, lane 1). Furthermore, the IL-17R ECD (SEQ ID NO:15) also precipitated in combination with IL-17 (SEQ ID NO:11) (lane 3). However, both IL-17B (SEQ ID NO:1) and IL-17C (SEQ ID NO:3) failed to compete for the binding of IL-17 (SEQ ID NO:11) for the labeled IL-17 receptor ECD (SEQ ID NO:15) (Fig. 11B, lane 15 and 16).

#### 10 Example 12: Fluorescence-Activated Cell Sorter (FACS) Analysis of Binding to THP-1 Cells

THP-1 cells ( $5 \times 10^5$ ) were pre-incubated in PBS containing 5% horse serum at 4°C for 30 minutes to block non-specific binding. IL-17 (SEQ ID NO:11), IL-17B.fc (SEQ ID NO:12), IL-17C.Fc (SEQ ID NO:13), or control Fc (1 µg each) were added and incubated with the THP-1 cells in a volume of 0.25 ml on ice for 1 hour. For the IL-17 binding experiment, primary anti hIL-17 antibody (1:100 dilution) and secondary goat anti-mouse antibody conjugated to FITC (Jackson Immunology Lab, 1:100 dilution) were added sequentially with 30-60 minutes incubation and extensive washes before each addition. For the Fc fusion proteins, the cells were stained with FITC conjugated goat anti-human IgG (Fc specific, Jackson Immunology Lab, 1:100 dilution). After thorough washes, a minimum of 5,000 cells were analyzed using a FACScan (Becton Dickinson).

The resulting of the above procedure was that both IL-17B (SEQ ID NO:12) and IL-17C (SEQ ID NO:13) Fc fusion proteins displayed binding to THP-1 cells compared with a control Fc fusion protein (Fig. 13).

#### 25 Example 13: Purification of PRO1031 or PRO1122 Polypeptides Using Specific Antibodies

Native or recombinant PRO1031 or PRO1122 polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO1031 or pro-PRO1122 polypeptide, mature PRO1031 or PRO1122 polypeptide, or pre-PRO1031 or pre-PRO1122 polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO1031 or PRO1122 polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO1031 or anti-PRO1122 antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE® (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO1031 or PRO1122 polypeptide by preparing a fraction of cells containing PRO1031 or PRO1122 polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble PRO1031 or PRO1122 polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO1031 or PRO1122 polypeptide-containing preparation is passed over the immunoaffinity column, the column is washed under conditions that allow the preferential absorbance of PRO1031 or PRO1122 polypeptide (e.g., high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody-PRO1031 or -PRO1122 polypeptide binding (e.g., a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and PRO1031 or PRO1122 polypeptide is collected.

#### Example 14: Drug Screening

This invention is particularly useful for screening compounds by using PRO1031 or PRO1122 polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The PRO1031 or PRO1122 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilized eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO1031 or PRO1122 polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO1031 or PRO1122 polypeptide or a fragment and the agent being tested. Alternatively,

one can examine the diminution in complex formation between the PRO1031 or PRO1122 polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO1031 or PRO1122 polypeptide-associated disease or disorder. These methods comprise contacting such an agent with a PRO1031 or PRO1122 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the PRO1031 or PRO1122 polypeptide or fragment, or (ii) for the presence of a complex between the PRO1031 or PRO1122 polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO1031 or PRO1122 polypeptide or fragment is typically labeled. After suitable incubation, free PRO1031 or PRO1122 polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO1031 or PRO1122 or to interfere with the PRO1031 or PRO1122 polypeptide/cell complex.

Another technique for drug screening provide high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO1031 or PRO1122 polypeptide, the peptide test compounds are reacted with PRO1031 or PRO1122 polypeptide and washed. Bound PRO1031 or PRO1122 is detected by methods well known in the art. Purified PRO1031 or PRO1122 polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO1031 or PRO1122 polypeptide specifically compete with a test compound for binding to PRO1031 or PRO1122 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO1031 or PRO1122 polypeptide.

30

#### Example 15: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO1031 or PRO1122 polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists, or inhibitors. Any of these examples can be

used to fashion drugs which are more active or stable forms of the PRO1031 or PRO1122 polypeptide or which enhance or interfere with the function of the PRO1031 or PRO1122 polypeptide *in vivo*. (c.f., Hodgson, *Bio/Technology* 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO1031 or PRO1122 polypeptide, or of a PRO1031 or PRO1122 polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO1031 or PRO1122 must be ascertained to elucidate the structure and to determine active sites(s) of the molecule. Less often, useful information regarding the structure of the PRO1031 or PRO1122 may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO1031 or PRO1122 polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, *Biochemistry* 31: 7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, *J. Biochem.* 113: 742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of anti anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO1031 or PRO1122 polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO1031 or PRO1122 polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place or in addition to x-ray crystallography.

#### Example 16: Articular cartilage Explant Assay

##### *Introduction:*

As mentioned previously, IL-17 is likely to play a role in the initiation or maintenance of the proinflammatory response. IL-17 is a cytokine expressed by CD4<sup>+</sup> T<sub>H</sub> cells and

induces the secretion of proinflammatory and hematopoietic cytokines (e.g., IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-8, GM-CSF. Aarvak *et al.*, *J. Immunol* 162: 1246-1251 (1999); Fossiez *et al.*, *J. Exp. Med.* 183: 2593-2603 (1996); Jovanovic *et al.*, *J. Immunol.* 160: 3513-3521 (1998) in a number of cell types including synoviocytes and macrophages. In the presence of IL-17, fibroblasts sustain the proliferation of CD34+ hematopoietic progenitors and induce their preferential maturation into neutrophils. As a result, IL-17 may constitute an early initiator of the T cell-dependent inflammatory reaction and be part of the cytokine network which bridges the immune system to hematopoiesis.

Expression of IL-17 has been found in the synovium of patients with rheumatoid arthritis, psoriatic arthritis, or osteoarthritis, but not in normal joint tissues. IL-17 can synergize with the monocyte-derived, proinflammatory cytokines IL-1 $\beta$  or TNF- $\alpha$  to induce IL-6 and GM-CSF. By acting directly on synoviocytes, IL-17 could enhance secretion of proinflammatory cytokines in vivo and thus exacerbate joint inflammation and destruction.

To further understand the possible role of IL-17, Applicants have tested the effects of IL-17 on cartilage matrix metabolism. In light of the known catabolic effects of nitric oxide (NO) on cartilage, and the existence of high levels of NO in arthritic joints, NO production was also measured.

#### *Methods:*

Articular cartilage explants: The metacarpophalangeal joint of a 4-6 month old female pigs was aseptically dissected, and articular cartilage is removed by free-hand slicing in a careful manner so as to avoid the underlying bone. The cartilage was minced and cultured in bulk for at least 24 hours in a humidified atmosphere of 95% air 5% CO<sub>2</sub> in serum free (SF) media (DME/F12 1:1) with 0.1% BSA and antibiotics. After washing three times, approximately 80 mg of articular cartilage was aliquoted into micronics tubes and incubated for at least 24 hours in the above SF media. Test proteins were then added at 1% either alone or in combination with IL-1 $\alpha$  (10 ng/ml)(SEQ ID NO:25). Media was harvested and changed at various timepoints (0, 24, 48, 72 hours) and assayed for proteoglycan content using the 1,9-dimethyl-methylene blue (DMB) colorimetric assay described in Farndale and Buttle, *Biochem. Biophys. Acta* 883: 173-177 (1985). After labeling (overnight) with <sup>35</sup>S-sulfur, the tubes were weighed to determine the amount of tissue. Following an overnight digestion, the amount of proteoglycan remaining in the tissue as well as proteoglycan synthesis (<sup>35</sup>S-incorporation) is determined.



Measurement of NO production: The assay is based on the principle that 2,3-diaminonaphthalene (DAN) reacts with nitrite under acidic conditions to form 1-(H)-naphthotriazole, a fluorescent product. As NO is quickly metabolized into nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), detection of nitrite, is one means of detecting (albeit undercounting) the actual NO produced. 10  $\mu\text{L}$  of DAN (0.05 mg. mL in 0.62M HCl) is added to 100  $\mu\text{L}$  of sample (cell culture supernatant), mixed, and incubated at room temperature for 10-20 minutes. Reaction is terminated with 5 mL of 2.8N NaOH. Formation of 2,3-diaminonaphthotriazole was measured using a Cytoflor fluorescent plate reader with excitation at 360 nm and emission read at 450 nm. For optimal measurement of fluorescent intensity, black plates with clear bottoms were used.

#### Results and Discussion:

IL-17 (SEQ ID NO:11) was observed to both increase the release of and decrease the synthesis of proteoglycans (Figure 13). Moreover, this effect was additive to the effect observed from IL-1 $\alpha$ . (Figure 13)(SEQ ID NO:25). The effects of IL-17 are not mediated by the production of nitric oxide, nor does inhibition of nitric oxide release augment matrix breakdown. UNQ561 (SEQ ID NO:3) increases matrix breakdown and inhibits matrix synthesis. Thus, expression of PRO1122 is likely to be associated with degenerative cartilagenous disorders. On the other hand, UNQ516 (SEQ ID NO:1) increases matrix synthesis and inhibits nitric oxide release by articular cartilage explants.

In conclusion, IL-17 likely contributes to loss of articular cartilage in arthritic joints, and thus inhibition of its activity might limit inflammation and cartilage destruction. IL-1 and IL-17 have similar yet distinct activities, due to their use of different receptors and overlapping downstream signaling mechanisms.

Given the findings of the potent catabolic effects of IL-17 on articular cartilage explants and the homology of UNQ516 and UNQ561 to IL-17, antagonists to any or all of these proteins may be useful for the treatment of inflammatory conditions and cartilage defects such as arthritis. However, our finding that UNQ 516 inhibits NO production and enhances matrix synthesis suggests that this protein and agonists thereof could have beneficial effects within the joint and may thus, in and of itself, be useful for the treatment of the above mentioned disorders.

Finally, it is well known that growth factors can have biphasic effects and that diseased tissue can respond differently than normal tissue to a given factor in vivo. For these reasons, antagonists or agonists (*e.g.* the proteins themselves) of UNQ 516, UNQ 561, or IL-17, may be useful for the treatment of inflammatory conditions and joint disorders such as arthritis.

\*\*\*\*\*

#### Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA USA 20110-2209 (ATCC):

| <u>Material</u> | <u>ATCC Dep. No.</u> | <u>Deposit Date</u> |
|-----------------|----------------------|---------------------|
| DNA59294-1381   | 209866               | 14 May 1998         |
| DNA62377-1381-1 | 203546               | 22 Dec. 1998        |

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC §122 and the Commissioner's rules pursuant thereto (including 37 CFR §1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect

of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of  
5 the appended claims.

WHAT IS CLAIMED IS:

1. Isolated nucleic acid comprising DNA having at least an 80% sequence identity to (a) a DNA molecule encoding a polypeptide selected from the group consisting of:
  - (1) a PRO1031 polypeptide comprising the sequence of amino acid residues 1 or 21 through 180, inclusive of Figure 1 (SEQ ID NO:1), and
  - (2) a PRO1132 polypeptide comprising the sequence of amino acid residues 1 or about 1 or about 19 through 197, inclusive; or
 (b) the complement of the DNA molecule of (a).
2. The nucleic acid of Claim 1, wherein said DNA comprises the sequence of corresponding nucleotide positions: (1) 42 to about 581, inclusive, of SEQ ID NO:2 or (2) 49 to about 640, inclusive, of SEQ ID NO:4.
3. The nucleic acid of Claim 1, wherein said DNA comprises the nucleotide selected from the group consisting of sequence of SEQ ID NO:2 and SEQ ID NO:4.
4. The isolated nucleic acid molecule of Claim 1 comprising a nucleotide sequence that encodes the sequence of amino acid selected from the group consisting of: (1) residues from 1 or about 21 to about 180 of Figure 1 (SEQ ID NO:1) and (2) residues from 1 or about 19 to about 197 of Figure 2 (SEQ ID NO:3).
5. Isolated nucleic acid comprising DNA having at least an 80% sequence identity to a DNA molecule (a) a DNA molecule encoding the same mature polypeptide encoded by the human protein cDNA selected from the group consisting of: (1) ATCC Deposit 209866 and (2) ATCC Deposit 203552; or (b) the complement of the DNA molecule of (a).
6. The isolated nucleic acid molecule of Claim 5 comprising DNA encoding the same mature polypeptide encoded by the human protein cDNA deposited with the ATCC under ATCC Deposit Number 209866 or 203552.
7. An isolated nucleic acid molecule comprising DNA which comprises at least about 80% sequence identity to (a) the full-length polypeptide coding sequence of the human

protein cDNA deposited with the ATCC under ATCC deposit numbers 209866 or 203552, or  
(b) the complement of the coding sequence of (a).

8. The isolated nucleic acid molecule of claim 7 comprising the full-length  
5 polypeptide coding sequence of the human protein cDNA deposited with the ATCC under  
ATCC Deposit Nos. 209866 or 203552.

9. An isolated nucleic acid molecule encoding a PRO1031 or PRO1122  
polypeptide comprising DNA that hybridizes to the complement of the nucleic acid sequence  
10 that encodes a polypeptide selected from the group consisting of: (1) amino acids 1 or about  
21 to about 180 of Figure 1 (SEQ ID NO:1); (2) amino acids 1 or about 19 to about 197 of  
Figure 3 (SEQ ID NO:3).

10. The isolated nucleic acid molecule of claim 9, wherein the nucleic acid that  
15 encodes (1) amino acids 1 or about 21 to about 180, inclusive, of Figure 1 (SEQ ID NO:1) or  
(2) amino acids 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID NO:3) comprises  
nucleotides (1) 42 or about 102 to about 581, inclusive, of Figure 2 (SEQ ID NO:2) or (2) 49  
or about 104 to about 640, inclusive, of Figure 4 (SEQ ID NO:4), respectively.

20 11. The isolated nucleic acid molecule of claim 9, wherein hybridization occurs  
under stringent hybridization and wash conditions.

12. An isolated nucleic acid molecule comprising (a) DNA encoding a  
polypeptide scoring at least 80% positives when compared to the sequence of amino acid  
25 residues selected from the group consisting of: (1) from 1 or about 21 to about 180, inclusive,  
of Figure 1 (SEQ ID NO:1); or (2) from 1 or about 19 to about 197, inclusive, of Figure 3  
(SEQ ID NO:3); or (b) the complement of the DNA of (a).

13. An isolated nucleic acid molecule comprising at least about 250 nucleotides in  
30 length and which is produced by hybridizing a test DNA under stringent hybridization  
conditions with (a) a DNA molecule which encodes a PRO1031 or PRO1122 polypeptide  
comprising a sequence of amino acid residues from 1 or about 21 to about 180, inclusive, of  
Figure 1 (SEQ ID NO:1); or from 1 or about 19 to about 197, inclusive, of Figure 3 (SEQ ID

NO:3), respectively, or (b) the complement of the DNA molecule of (a), and isolating the test DNA molecule.

14. The isolated nucleic acid molecule of claim 13, which has at least about 80%  
5 sequence identity to (a) or (b).

15. A vector comprising the nucleic acid molecule of any of Claims 1 to 14.

16. The vector of Claim 15, wherein said nucleic acid molecule is operably linked to  
10 control sequences recognized by a host cell transformed with a the vector.

17. A nucleic acid molecule deposited with the ATCC number under accession number 209866 or 203553.

15 18. A host cell comprising the vector of Claim 15.

19. The host cell of Claim 18, wherein said cell is a CHO cell.

20. The host cell of Claim 18, wherein said cell is an *E. coli*.

20

21. The host cell of Claim 18, wherein said cell is a yeast cell.

22. A process for procuring a PRO1031 or PRO1122 polypeptide comprising culturing the host cell of Claim 18 under conditions suitable for expression of said PRO1031 or  
25 PRO1122 polypeptide and recovering said PRO1031 or PRO1122 polypeptide from the cell culture.

23. An isolated polypeptide comprising an amino acid sequence comprising at least about 80% sequence identity to the sequence of amino acid residues selected from the group  
30 consisting of: (1) a PRO1031 polypeptide comprising residues 1 or about 21 to about 180 of Figure 1 (SEQ ID NO:1), and (2) a PRO1122 polypeptide comprising residues 1 or about 19 to about 197 of Figure 3 (SEQ ID NO:3).

24. The isolated PRO1031 or PRO1122 polypeptide of claim 23 comprising amino acid residues 1 or about 21 to about 180 of Figure 1 (SEQ ID NO:1) or 1 or about 19 to about 197 of Figure 3 (SEQ ID NO:3), respectively.

5 25. An isolated PRO1031 or PRO1122 polypeptide having at least about 80% sequence identity to the polypeptide encoded by the cDNA insert of the vector deposited with the ATCC as ATCC Deposit No. 209866 or 203552, respectively.

26. The isolated PRO1031 or PRO1122 polypeptide of Claim 25 which is encoded by the  
10 cDNA insert of the vector deposited with the ATCC as ATCC Deposit No. 209866 or 203552, respectively.

27. An isolated PRO1031 or PRO1122 polypeptide scoring at least 80% positives when compared to the sequence of amino acids from about 1 or about 21 to about 180 of Figure 1  
15 (SEQ ID NO:1) or 1 or about 19 to about 197 of Figure 3 (SEQ ID NO:3), respectively.

28. An isolated PRO1031 or PRO1122 polypeptide comprising the sequence of amino acid residues from 1 or about 21 to about 180 of Figure 1 (SEQ ID NO:1), or 1 or about 19 to about 197 of Figure 3 (SEQ ID NO:3), respectively, or a fragment thereof sufficient to  
20 provide a binding site for an anti-PRO1031 or anti-PRO1122 antibody, respectively.

29. An isolated polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1031 or PRO1122 polypeptide comprising the sequence of amino acid residues from 1 or about 21 to about 180 of Figure 1  
25 (SEQ ID NO:1), or 1 or about 19 to about 197 of Figure 3 (SEQ ID NO:3), respectively; or (b) the complement of the DNA molecule of (a); (ii) culturing a host cell comprising the said test DNA molecule under conditions suitable for the expression of said polypeptide, and (iii) recovering said polypeptide from the cell culture.

30. The isolated polypeptide of Claim 29, wherein said test DNA has at least about 80% sequence identity to (a) or (b).

31. A chimeric molecule comprising a PRO1031 or PRO1122 polypeptide fused to a heterologous amino acid sequence.

32. The chimeric molecule of Claim 31, wherein said heterologous amino acid sequence is an epitope tag sequence.
- 5 33. The chimeric molecule of Claim 31, wherein said heterologous amino acid sequence is an Fc region of an immunoglobulin.
34. An antibody which specifically binds to a PRO1031 or PRO1122 polypeptide.
- 10 35. The antibody of Claim 34, where said antibody is a monoclonal antibody.
36. The antibody of Claim 34, wherein said antibody is a humanized antibody.
37. An agonist to a PRO1031, PRO1122 or IL-17 polypeptide.
- 15 38. An antagonist to a PRO1031, PRO1122 or IL-17 polypeptide.
39. A composition comprising a therapeutically effective amount of an active agent selected from the group consisting of: (a) a PRO1031 or PRO1122 polypeptide, (b) an  
20 agonist to a PRO1031 or PRO1122 polypeptide, (c) an antagonist to a PRO1031 or PRO1122 polypeptide, and (d) an anti-PRO1031 or anti-PRO1122 antibody; in combination with a pharmaceutically acceptable carrier.
40. A method of treating a degenerative cartilaginous disorder by administration of a  
25 therapeutically effective amount of a PRO1031 or PRO1122 polypeptide, agonist, or antagonist thereof to a mammal suffering from said disorder.
41. A method of diagnosing a degenerative cartilaginous disorder by: (1) culturing test cells or tissues expressing PRO1031 or PRO1122; (2) administering a compound which can  
30 inhibit PRO1031 or PRO1122 modulated signaling; and (3) measuring the PRO1031 or PRO1122 mediated phenotypic effects in the test cells or tissues.



42. An article of manufacture comprising a container, label and therapeutically effective amount of PRO1031, PRO1122, agonist or antagonist thereof in combination with a pharmaceutically-effective carrier.

Abstract of the Disclosure

The present invention is directed to novel polypeptides having sequence identity with  
5 IL-17 and to nucleic acid molecules encoding those polypeptides. Also provided herein are  
vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide  
molecules comprising the polypeptides of the present invention fused to heterologous  
polypeptide sequences, antibodies which bind to the polypeptides of the present invention  
and to methods for producing the polypeptides of the present invention. Further provided  
10 herein are methods for treating degenerative cartilaginous disorders.

## Sequence Listing

## Sequence Listing

<110> Chen, Jian  
 5 Filvaroff, Ellen  
 Goddard, Audrey  
 Gurney, Austin  
 Li, Hanzhong  
 Wood, William I.  
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 <120> IL-17 HOMOLOGOUS POLYPEPTIDES AND THERAPEUTIC USES  
 THEREOF  
 <130> P1381-R1  
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 <141> 1999-05-14  
 <150> US 60/085,579  
 <151> 1998-05-15  
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 Gly Gln Gly Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln Val  
 35 40 45  
 40 Pro Leu Asp Leu Val Ser Arg Met Lys Pro Tyr Ala Arg Met Glu  
 50 55 60  
 Glu Tyr Glu Arg Asn Ile Glu Glu Met Val Ala Gln Leu Arg Asn  
 45 65 70 75  
 Ser Ser Glu Leu Ala Gln Arg Lys Cys Glu Val Asn Leu Gln Leu  
 80 85 90

|    |  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
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|    | Trp  | Met | Ser | Asn | Lys | Arg | Ser | Leu | Ser | Pro | Trp | Gly | Tyr | Ser | Ile |  |
|    |  |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
| 5  | Asn  | His | Asp | Pro | Ser | Arg | Ile | Pro | Val | Asp | Leu | Pro | Glu | Ala | Arg |  |
|    |  |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
|    | Cys  | Leu | Cys | Leu | Gly | Cys | Val | Asn | Pro | Phe | Thr | Met | Gln | Glu | Asp |  |
|    |  |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
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|    |  |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |
|    | Arg  | Arg | Leu | Cys | Pro | Pro | Pro | Pro | Arg | Thr | Gly | Pro | Cys | Arg | Gln |  |
| 15 |  |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
|    | Arg  | Ala | Val | Met | Glu | Thr | Ile | Ala | Val | Gly | Cys | Thr | Cys | Ile | Phe |  |
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|    | cctcacaacc tgctgtttct tcttaccatt tccatcttcc tggggctggg 100 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 30 | ccagcccagg agccccaaaa gcaagaggaa ggggcaaggg cggcctgggc 150 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | ccctggcccc tggccctcac caggtgccac tggacctggt gtcacggatg 200 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | aaaccgtatg cccgcatgga ggagtatgag aggaacatcg aggagatggt 250 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 35 | ggcccagctg aggaacagct cagagctggc ccagagaaag tgtgaggtca 300 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | acttgcagct gtggatgtcc aacaagagga gctgtctcc ctggggctac 350  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 40 | agcatcaacc acgaccccag ccgtatcccc gtggacctgc cggaggcacg 400 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | gtgcctgtgt ctgggctgtg tgaaccctt caccatgcag gaggaccgca 450  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | gcatggtgag cgtgccggtg ttcagccagg ttctgtgcg ccgccgcctc 500  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 45 | tgcccggcac cgccccgcac agggccttgc cgccagcgcg cagtcatgga 550 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | gaccatcgct gtgggctgca cctgcatctt ctgaatcacc tggcccagaa 600 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |

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<212> PRT

<213> Homo sapiens

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<400> 3

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| Met | Thr | Leu | Leu | Pro | Gly | Leu | Leu | Phe | Leu | Thr | Trp | Leu | His | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

15

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Leu | Ala | His | His | Asp | Pro | Ser | Leu | Arg | Gly | His | Pro | His | Ser |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Gly | Thr | Pro | His | Cys | Tyr | Ser | Ala | Glu | Glu | Leu | Pro | Leu | Gly |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

20

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Pro | Pro | His | Leu | Leu | Ala | Arg | Gly | Ala | Lys | Trp | Gly | Gln |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Leu | Pro | Val | Ala | Leu | Val | Ser | Ser | Leu | Glu | Ala | Ala | Ser | His |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |

25

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gly | Arg | His | Glu | Arg | Pro | Ser | Ala | Thr | Thr | Gln | Cys | Pro | Val |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |

30

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Arg | Pro | Glu | Glu | Val | Leu | Glu | Ala | Asp | Thr | His | Gln | Arg | Ser |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Ser | Pro | Trp | Arg | Tyr | Arg | Val | Asp | Thr | Asp | Glu | Asp | Arg | Tyr |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |

35

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gln | Lys | Leu | Ala | Phe | Ala | Glu | Cys | Leu | Cys | Arg | Gly | Cys | Ile |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ala | Arg | Thr | Gly | Arg | Glu | Thr | Ala | Ala | Leu | Asn | Ser | Val | Arg |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |

40

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | Gln | Ser | Leu | Leu | Val | Leu | Arg | Arg | Arg | Pro | Cys | Ser | Arg |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |

45

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Gly | Ser | Gly | Leu | Pro | Thr | Pro | Gly | Ala | Phe | Ala | Phe | His | Thr |
|     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |

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Ser Val  
 197

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&lt;211&gt; 1047

&lt;212&gt; DNA

10 &lt;213&gt; Homo sapiens

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 20 tgctggctcg aggtgccaaag tgggggcagg ctttgctgt agccctgggtg 250  
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 25 tacgaccag tgcccgggtgc tgcggccgga ggaggtgttg gaggcagaca 350  
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20 tggcctggcc tcaccaggtg ccaactggacc tgggtgtcacg gatgaaaccg 200

tatgcccgca tggaggagta tgagaggaac atcgaggaga tgggtggcca 250

25 gctgaggaac agtcanaag ctggcccaga gaaagtgtga ggtcaacttg 300

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Met Val Asn Leu Asn Ile His Asn Arg Asn Thr Asn Thr Asn Pro  
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40 Lys Arg Ser Ser Asp Tyr Tyr Asn Arg Ser Thr Ser Pro Trp Asn  
 65 70 75

Leu His Arg Asn Glu Asp Pro Glu Arg Tyr Pro Ser Val Ile Trp  
 80 85 90

Glu Ala Lys Cys Arg His Leu Gly Cys Ile Asn Ala Asp Gly Asn  
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Val Asp Tyr His Met Asn Ser Val Pro Ile Gln Gln Glu Ile Leu  
 110. 115 120

5 Val Leu Arg Arg Glu Pro Pro His Cys Pro Asn Ser Phe Arg Leu  
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Gly Gln Gly Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln Val  
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Pro Leu Asp Leu Val Ser Arg Met Lys Pro Tyr Ala Arg Met Glu  
 50 55 60

35 Glu Tyr Glu Arg Asn Ile Glu Glu Met Val Ala Gln Leu Arg Asn  
 65 70 75

Ser Ser Glu Leu Ala Gln Arg Lys Cys Glu Val Asn Leu Gln Leu  
 80 85 90

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Asn His Asp Pro Ser Arg Ile Pro Val Asp Leu Pro Glu Ala Arg  
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Cys Leu Cys Leu Gly Cys Val Asn Pro Phe Thr Met Gln Glu Asp  
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| 5  | Arg Arg Leu Cys Pro Pro Pro Pro Arg Thr Gly Pro Cys Arg Gln | 155 | 160 | 165 |
|    | Arg Ala Val Met Glu Thr Ile Ala Val Gly Cys Thr Cys Ile Phe | 170 | 175 | 180 |
| 10 | Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu | 185 | 190 | 195 |
|    | Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp | 200 | 205 | 210 |
| 15 | Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val | 215 | 220 | 225 |
|    | Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val | 230 | 235 | 240 |
|    | Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu | 245 | 250 | 255 |
| 25 | Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu | 260 | 265 | 270 |
|    | His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser | 275 | 280 | 285 |
| 30 | Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala | 290 | 295 | 300 |
|    | Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser | 305 | 310 | 315 |
|    | Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val | 320 | 325 | 330 |
| 40 | Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn | 335 | 340 | 345 |
|    | Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp | 350 | 355 | 360 |
| 45 | Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys | 365 | 370 | 375 |

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
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Pro Gly Lys  
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 50 55 60

Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His  
 65 70 75

35 Arg Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln Cys Pro Val  
 80 85 90

Leu Arg Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln Arg Ser  
 95 100 105

40 Ile Ser Pro Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg Tyr  
 110 115 120

45 Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile  
 125 130 135

Asp Ala Arg Thr Gly Arg Glu Thr Ala Ala Leu Asn Ser Val Arg  
 140 145 150

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Leu | Leu | Gln | Ser | Leu | Leu | Val | Leu | Arg | Arg | Arg | Pro | Cys | Ser | Arg |  |
|    |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
| 5  | Asp | Gly | Ser | Gly | Leu | Pro | Thr | Pro | Gly | Ala | Phe | Ala | Phe | His | Thr |  |
|    |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
|    | Glu | Phe | Ile | His | Val | Pro | Val | Gly | Cys | Thr | Cys | Val | Leu | Pro | Arg |  |
|    |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |
| 10 | Ser | Val | Pro | Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro |  |
|    |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
|    | Glu | Leu | Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro |  |
| 15 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |  |
|    | Lys | Asp | Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val |  |
|    |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| 20 | Val | Val | Asp | Val | Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp |  |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |  |
|    | Tyr | Val | Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg |  |
|    |     |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |  |
| 25 | Glu | Glu | Gln | Tyr | Asn | Ser | Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr |  |
|    |     |     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |  |
|    | Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys |  |
| 30 |     |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |  |
|    | Val | Ser | Asn | Lys | Ala | Leu | Pro | Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser |  |
|    |     |     |     |     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |  |
| 35 | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr | Leu | Pro |  |
|    |     |     |     |     | 320 |     |     |     |     | 325 |     |     |     |     | 330 |  |
|    | Pro | Ser | Arg | Glu | Glu | Met | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr | Cys |  |
|    |     |     |     |     | 335 |     |     |     |     | 340 |     |     |     |     | 345 |  |
| 40 | Leu | Val | Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu |  |
|    |     |     |     |     | 350 |     |     |     |     | 355 |     |     |     |     | 360 |  |
|    | Ser | Asn | Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr | Pro | Pro | Val |  |
| 45 |     |     |     |     | 365 |     |     |     |     | 370 |     |     |     |     | 375 |  |
|    | Leu | Asp | Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu | Thr | Val |  |
|    |     |     |     |     | 380 |     |     |     |     | 385 |     |     |     |     | 390 |  |

|    |                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|----|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
|    | Asp                | Lys | Ser | Arg | Trp | Gln | Gln | Gly | Asn | Val | Phe | Ser | Cys | Ser | Val | 395 | 400 | 405 |    |
| 5  | Met                | His | Glu | Ala | Leu | His | Asn | His | Tyr | Thr | Gln | Lys | Ser | Leu | Ser | 410 | 415 | 420 |    |
|    | Leu                | Ser | Pro | Gly | Lys | 425 |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 10 |                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | <210> 14           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | <211> 212          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | <212> PRT          |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | <213> Homo sapiens |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
| 15 |                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | <400> 14           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|    | Met                | Asn | Ser | Phe | Ser | Thr | Ser | Ala | Phe | Gly | Pro | Val | Ala | Phe | Ser | 1   | 5   | 10  | 15 |
| 20 | Leu                | Gly | Leu | Leu | Leu | Val | Leu | Pro | Ala | Ala | Phe | Pro | Ala | Pro | Val | 20  | 25  | 30  |    |
|    | Pro                | Pro | Gly | Glu | Asp | Ser | Lys | Asp | Val | Ala | Ala | Pro | His | Arg | Gln | 35  | 40  | 45  |    |
| 25 | Pro                | Leu | Thr | Ser | Ser | Glu | Arg | Ile | Asp | Lys | Gln | Ile | Arg | Tyr | Ile | 50  | 55  | 60  |    |
| 30 | Leu                | Asp | Gly | Ile | Ser | Ala | Leu | Arg | Lys | Glu | Thr | Cys | Asn | Lys | Ser | 65  | 70  | 75  |    |
|    | Asn                | Met | Cys | Glu | Ser | Ser | Lys | Glu | Ala | Leu | Ala | Glu | Asn | Asn | Leu | 80  | 85  | 90  |    |
| 35 | Asn                | Leu | Pro | Lys | Met | Ala | Glu | Lys | Asp | Gly | Cys | Phe | Gln | Ser | Gly | 95  | 100 | 105 |    |
|    | Phe                | Asn | Glu | Glu | Thr | Cys | Leu | Val | Lys | Ile | Ile | Thr | Gly | Leu | Leu | 110 | 115 | 120 |    |
| 40 | Glu                | Phe | Glu | Val | Tyr | Leu | Glu | Tyr | Leu | Gln | Asn | Arg | Phe | Glu | Ser | 125 | 130 | 135 |    |
| 45 | Ser                | Glu | Glu | Gln | Ala | Arg | Ala | Val | Gln | Met | Ser | Thr | Lys | Val | Leu | 140 | 145 | 150 |    |
|    | Ile                | Gln | Phe | Leu | Gln | Lys | Lys | Ala | Lys | Asn | Leu | Asp | Ala | Ile | Thr | 155 | 160 | 165 |    |

|    |       |              |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Thr   | Pro          | Asp | Pro | Thr | Thr | Asn | Ala | Ser | Leu | Leu | Thr | Lys | Leu | Gln |  |
|    |       |              |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
| 5  | Ala   | Gln          | Asn | Gln | Trp | Leu | Gln | Asp | Met | Thr | Thr | His | Leu | Ile | Leu |  |
|    |       |              |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |
|    | Arg   | Ser          | Phe | Lys | Glu | Phe | Leu | Gln | Ser | Ser | Leu | Arg | Ala | Leu | Arg |  |
|    |       |              |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
| 10 | Gln   | Met          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    |       | 212          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <210> | 15           |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 15 | <211> | 320          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <212> | PRT          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <213> | Homo sapiens |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <400> | 15           |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 20 | Met   | Gly          | Ala | Ala | Arg | Ser | Pro | Pro | Ser | Ala | Val | Pro | Gly | Pro | Leu |  |
|    | 1     |              |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |  |
|    | Leu   | Gly          | Leu | Leu | Leu | Leu | Leu | Leu | Gly | Val | Leu | Ala | Pro | Gly | Gly |  |
|    |       |              |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |  |
| 25 | Ala   | Ser          | Leu | Arg | Leu | Leu | Asp | His | Arg | Ala | Leu | Val | Cys | Ser | Gln |  |
|    |       |              |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |  |
|    | Pro   | Gly          | Leu | Asn | Cys | Thr | Val | Lys | Asn | Ser | Thr | Cys | Leu | Asp | Asp |  |
| 30 |       |              |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |  |
|    | Ser   | Trp          | Ile | His | Pro | Arg | Asn | Leu | Thr | Pro | Ser | Ser | Pro | Lys | Asp |  |
|    |       |              |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |  |
| 35 | Leu   | Gln          | Ile | Gln | Leu | His | Phe | Ala | His | Thr | Gln | Gln | Gly | Asp | Leu |  |
|    |       |              |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |  |
|    | Phe   | Pro          | Val | Ala | His | Ile | Glu | Trp | Thr | Leu | Gln | Thr | Asp | Ala | Ser |  |
|    |       |              |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
| 40 | Ile   | Leu          | Tyr | Leu | Glu | Gly | Ala | Glu | Leu | Ser | Val | Leu | Gln | Leu | Asn |  |
|    |       |              |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
|    | Thr   | Asn          | Glu | Arg | Leu | Cys | Val | Arg | Phe | Glu | Phe | Leu | Ser | Lys | Leu |  |
| 45 |       |              |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
|    | Arg   | His          | His | His | Arg | Arg | Trp | Arg | Phe | Thr | Phe | Ser | His | Phe | Val |  |
|    |       |              |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Val | Asp | Pro | Asp | Gln | Glu | Tyr | Glu | Val | Thr | Val | His | His | Leu | Pro |  |
|    |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
| 5  | Lys | Pro | Ile | Pro | Asp | Gly | Asp | Pro | Asn | His | Gln | Ser | Lys | Asn | Phe |  |
|    |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
|    | Leu | Val | Pro | Asp | Cys | Glu | His | Ala | Arg | Met | Lys | Val | Thr | Thr | Pro |  |
|    |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |
| 10 | Cys | Met | Ser | Ser | Gly | Ser | Leu | Trp | Asp | Pro | Asn | Ile | Thr | Val | Glu |  |
|    |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
|    | Thr | Leu | Glu | Ala | His | Gln | Leu | Arg | Val | Ser | Phe | Thr | Leu | Trp | Asn |  |
| 15 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |  |
|    | Glu | Ser | Thr | His | Tyr | Gln | Ile | Leu | Leu | Thr | Ser | Phe | Pro | His | Met |  |
|    |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| 20 | Glu | Asn | His | Ser | Cys | Phe | Glu | His | Met | His | His | Ile | Pro | Ala | Pro |  |
|    |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |  |
|    | Arg | Pro | Glu | Glu | Phe | His | Gln | Arg | Ser | Asn | Val | Thr | Leu | Thr | Leu |  |
|    |     |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |  |
| 25 | Arg | Asn | Leu | Lys | Gly | Cys | Cys | Arg | His | Gln | Val | Gln | Ile | Gln | Pro |  |
|    |     |     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |  |
|    | Phe | Phe | Ser | Ser | Cys | Leu | Asn | Asp | Cys | Leu | Arg | His | Ser | Ala | Thr |  |
| 30 |     |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |  |
|    | Val | Ser | Cys | Pro | Glu | Met | Pro | Asp | Thr | Pro | Glu | Pro | Ile | Pro | Asp |  |
|    |     |     |     |     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |  |
| 35 | Tyr | Met | Pro | Leu | Trp |     |     |     |     |     |     |     |     |     |     |  |
|    |     |     |     |     | 320 |     |     |     |     |     |     |     |     |     |     |  |

&lt;210&gt; 16

&lt;211&gt; 543

40 &lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 16

45 atggactggc ctcacaacct gctgtttctt cttaccattt ccatcttcct 50

ggggctgggc cagcccagga gcccacaaag caagaggaag gggcaagggc 100

ggcctgggccc cctggcccct ggccctcacc aggtgccact ggacctggtg 150



tcacggatga aaccgtatgc ccgcatggag gagtatgaga ggaacatcga 200  
 ggagatggtg gccagctga ggaacagctc agagctggcc cagagaaagt 250  
 5 gtgaggtcaa cttgcagctg tggatgtcca acaagaggag cctgtctccc 300  
 tggggctaca gcatcaacca cgaccccagc cgtatccccg tggacctgcc 350  
 10 ggaggcacgg tgctgtgtc tgggctgtgt gaacccttc accatgcagg 400  
 aggaccgcag catggtgagc gtgccggtgt tcagccaggt tctgtgcgc 450  
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<210> 17

<211> 594

20 <212> DNA

<213> Homo sapiens

<400> 17

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 25 ggcccacat gaccctccc tcagggggca ccccccacagt cacggtaccc 100  
 cacactgcta ctcggtgag gaactgcccc tcggccaggc cccccacac 150  
 30 ctgctggctc gaggtgcaa gtgggggcag gctttgcctg tagccctggt 200  
 gtccagcctg gaggcagcaa gccacagggg gaggcacgag aggccctcag 250  
 ctacgacca gtgcccgggt ctgcggccgg aggaggtgtt ggaggcagac 300  
 35 acccaccagc gctccatctc accctggaga taccgtgtgg acacggatga 350  
 ggaccgctat ccacagaagc tggccttcgc cgagtgcctg tgcagaggct 400  
 40 gtatcgatgc acggacgggc cgcgagacag ctgcgctcaa ctccgtgcgg 450  
 ctgctccaga gcctgctggt gctgcgccgc cggccctgct cccgcgacgg 500  
 ctcggggctc cccacacctg gggcctttgc ctccacacc gagttcatcc 550  
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<210> 18

<211> 9  
 <212> PRT  
 <213> Artificial

5 <220>  
 <223> Artificial sequence 1-9

<400> 18  
 Gly His His His His His His His His  
 10 1 5 9

<210> 19  
 <211> 157  
 <212> PRT  
 15 <213> Homo sapiens

<400> 19  
 Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His  
 1 5 10 15  
 20 Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn  
 20 25 30  
 Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp  
 25 35 40 45  
 Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser  
 50 55 60  
 30 Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu  
 65 70 75  
 Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys  
 80 85 90  
 35 Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr  
 95 100 105  
 Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu  
 40 110 115 120  
 Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu  
 125 130 135  
 45 Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val  
 140 145 150  
 Tyr Phe Gly Ile Ile Ala Leu

155 157

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 5 <212> DNA  
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 ctgtacctcg aggtgcaga g 21  
  
 <210> 21  
 15 <211> 58  
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 25 ttagtcc 58  
  
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 30 <213> Homo sapiens  
  
 <400> 22  
 Met Gly Ala Ala Arg Ser Pro Pro Ser Ala Val Pro Gly Pro Leu  
 1 5 10 15  
 35 Leu Gly Leu Leu Leu Leu Leu Leu Gly Val Leu Ala Pro Gly Gly  
 20 25 30  
 Ala Ser Leu Arg Leu Leu Asp His Arg Ala Leu Val Cys Ser Gln  
 40 35 40 45  
 Pro Gly Leu Asn Cys Thr Val Lys Asn Ser Thr Cys Leu Asp Asp  
 50 55 60  
 45 Ser Trp Ile His Pro Arg Asn Leu Thr Pro Ser Ser Pro Lys Asp  
 65 70 75

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Leu | Gln | Ile | Gln | Leu | His | Phe | Ala | His | Thr | Gln | Gln | Gly | Asp | Leu |  |
|    |     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |  |
| 5  | Phe | Pro | Val | Ala | His | Ile | Glu | Trp | Thr | Leu | Gln | Thr | Asp | Ala | Ser |  |
|    |     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
|    | Ile | Leu | Tyr | Leu | Glu | Gly | Ala | Glu | Leu | Ser | Val | Leu | Gln | Leu | Asn |  |
|    |     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
| 10 | Thr | Asn | Glu | Arg | Leu | Cys | Val | Arg | Phe | Glu | Phe | Leu | Ser | Lys | Leu |  |
|    |     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
|    | Arg | His | His | His | Arg | Arg | Trp | Arg | Phe | Thr | Phe | Ser | His | Phe | Val |  |
|    |     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |
| 15 | Val | Asp | Pro | Asp | Gln | Glu | Tyr | Glu | Val | Thr | Val | His | His | Leu | Pro |  |
|    |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
|    | Lys | Pro | Ile | Pro | Asp | Gly | Asp | Pro | Asn | His | Gln | Ser | Lys | Asn | Phe |  |
| 20 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
|    | Leu | Val | Pro | Asp | Cys | Glu | His | Ala | Arg | Met | Lys | Val | Thr | Thr | Pro |  |
|    |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |
| 25 | Cys | Met | Ser | Ser | Gly | Ser | Leu | Trp | Asp | Pro | Asn | Ile | Thr | Val | Glu |  |
|    |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
|    | Thr | Leu | Glu | Ala | His | Gln | Leu | Arg | Val | Ser | Phe | Thr | Leu | Trp | Asn |  |
|    |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |  |
| 30 | Glu | Ser | Thr | His | Tyr | Gln | Ile | Leu | Leu | Thr | Ser | Phe | Pro | His | Met |  |
|    |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
|    | Glu | Asn | His | Ser | Cys | Phe | Glu | His | Met | His | His | Ile | Pro | Ala | Pro |  |
| 35 |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |  |
|    | Arg | Pro | Glu | Glu | Phe | His | Gln | Arg | Ser | Asn | Val | Thr | Leu | Thr | Leu |  |
|    |     |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |  |
| 40 | Arg | Asn | Leu | Lys | Gly | Cys | Cys | Arg | His | Gln | Val | Gln | Ile | Gln | Pro |  |
|    |     |     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |  |
|    | Phe | Phe | Ser | Ser | Cys | Leu | Asn | Asp | Cys | Leu | Arg | His | Ser | Ala | Thr |  |
|    |     |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |  |
| 45 | Val | Ser | Cys | Pro | Glu | Met | Pro | Asp | Thr | Pro | Glu | Pro | Ile | Pro | Asp |  |
|    |     |     |     |     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |  |

Tyr Met Pro Leu Trp His His His His His His His His  
320 325 328

<210> 23

5 <211> 175

<212> PRT

<213> Artificial

$\langle 220 \rangle$

10 <223> Artificial sequence 1-175

<400> 23

Ile Phe Leu Gly Leu Gly Gln Pro Arg Ser Pro Lys Ser Lys Arg  
1 5 10 15

15

Lys Gly Gln Gly Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln  
20 25 30

20

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Pro | Leu | Asp | Leu | Val | Ser | Arg | Met | Lys | Pro | Tyr | Ala | Arg | Met |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

Glu Glu Tyr Glu Arg Asn Ile Glu Glu Met Val Ala Gln Leu Arg  
50 55 60

25

Asn Ser Ser Glu Leu Ala Gln Arg Lys Cys Glu Val Asn Leu Gln  
65 70 75

Leu Trp Met Ser Asn Lys Arg Ser Leu Ser Pro Trp Gly Tyr Ser  
80 85 90

30

Ile Asn His Asp Pro Ser Arg Ile Pro Val Asp Leu Pro Glu Ala  
95 100 105

35

Arg Cys Leu Cys Leu Gly Cys Val Asn Pro Phe Thr Met Gln Glu  
110 115 120

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Arg | Ser | Met | Val | Ser | Val | Pro | Val | Phe | Ser | Gln | Val | Pro | Val |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

40

Arg Arg Arg Leu Cys Pro Pro Pro Pro Arg Thr Gly Pro Cys Arg  
140 145 150

Gln Arg Ala Val Met Glu Thr Ile Ala Val Gly Cys Thr Cys Ile  
155 160 165

45

Phe Gly His His His His His His His His  
170 175

<210> 24  
 <211> 206  
 <212> PRT  
 <213> Artificial

5

<220>  
 <223> Artificial sequence 1-206

<400> 24

|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| 10 | Met | Thr | Leu | Leu | Pro | Gly | Leu | Leu | Phe | Leu | Thr | Trp | Leu | His | Thr |  |
|    | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |  |
|    | Cys | Leu | Ala | His | His | Asp | Pro | Ser | Leu | Arg | Gly | His | Pro | His | Ser |  |
|    |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |  |
| 15 | His | Gly | Thr | Pro | His | Cys | Tyr | Ser | Ala | Glu | Glu | Leu | Pro | Leu | Gly |  |
|    |     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |  |
|    | Gln | Ala | Pro | Pro | His | Leu | Leu | Ala | Arg | Gly | Ala | Lys | Trp | Gly | Gln |  |
| 20 |     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |  |
|    | Ala | Leu | Pro | Val | Ala | Leu | Val | Ser | Ser | Leu | Glu | Ala | Ala | Ser | His |  |
|    |     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |  |
| 25 | Arg | Gly | Arg | His | Glu | Arg | Pro | Ser | Ala | Thr | Thr | Gln | Cys | Pro | Val |  |
|    |     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |  |
|    | Leu | Arg | Pro | Glu | Glu | Val | Leu | Glu | Ala | Asp | Thr | His | Gln | Arg | Ser |  |
|    |     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
| 30 | Ile | Ser | Pro | Trp | Arg | Tyr | Arg | Val | Asp | Thr | Asp | Glu | Asp | Arg | Tyr |  |
|    |     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
|    | Pro | Gln | Lys | Leu | Ala | Phe | Ala | Glu | Cys | Leu | Cys | Arg | Gly | Cys | Ile |  |
| 35 |     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
|    | Asp | Ala | Arg | Thr | Gly | Arg | Glu | Thr | Ala | Ala | Leu | Asn | Ser | Val | Arg |  |
|    |     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |
| 40 | Leu | Leu | Gln | Ser | Leu | Leu | Val | Leu | Arg | Arg | Arg | Pro | Cys | Ser | Arg |  |
|    |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
|    | Asp | Gly | Ser | Gly | Leu | Pro | Thr | Pro | Gly | Ala | Phe | Ala | Phe | His | Thr |  |
|    |     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
| 45 | Glu | Phe | Ile | His | Val | Pro | Val | Gly | Cys | Thr | Cys | Val | Leu | Pro | Arg |  |
|    |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |

Ser Val Gly His His His His His His His His  
 200 205 206

<210> 25  
 5 <211> 271  
 <212> PRT  
 <213> Homo sapiens

<400> 25  
 10 Met Ala Lys Val Pro Asp Met Phe Glu Asp Leu Lys Asn Cys Tyr  
 1 5 10 15  
 Ser Glu Asn Glu Glu Asp Ser Ser Ser Ile Asp His Leu Ser Leu  
 20 25 30  
 15 Asn Gln Lys Ser Phe Tyr His Val Ser Tyr Gly Pro Leu His Glu  
 35 40 45  
 Gly Cys Met Asp Gln Ser Val Ser Leu Ser Ile Ser Glu Thr Ser  
 20 50 55 60  
 Lys Thr Ser Lys Leu Thr Phe Lys Glu Ser Met Val Val Val Ala  
 65 70 75  
 25 Thr Asn Gly Lys Val Leu Lys Lys Arg Arg Leu Ser Leu Ser Gln  
 80 85 90  
 Ser Ile Thr Asp Asp Asp Leu Glu Ala Ile Ala Asn Asp Ser Glu  
 95 100 105  
 30 Glu Glu Ile Ile Lys Pro Arg Ser Ala Pro Phe Ser Phe Leu Ser  
 110 115 120  
 Asn Val Lys Tyr Asn Phe Met Arg Ile Ile Lys Tyr Glu Phe Ile  
 35 125 130 135  
 Leu Asn Asp Ala Leu Asn Gln Ser Ile Ile Arg Ala Asn Asp Gln  
 140 145 150  
 40 Tyr Leu Thr Ala Ala Ala Leu His Asn Leu Asp Glu Ala Val Lys  
 155 160 165  
 Phe Asp Met Gly Ala Tyr Lys Ser Ser Lys Asp Asp Ala Lys Ile  
 170 175 180  
 45 Thr Val Ile Leu Arg Ile Ser Lys Thr Gln Leu Tyr Val Thr Ala  
 185 190 195

|    |       |              |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|----|-------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
|    | Gln   | Asp          | Glu | Asp | Gln | Pro | Val | Leu | Leu | Lys | Glu | Met | Pro | Glu | Ile |  |
|    |       |              |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
| 5  | Pro   | Lys          | Thr | Ile | Thr | Gly | Ser | Glu | Thr | Asn | Leu | Leu | Phe | Phe | Trp |  |
|    |       |              |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |  |
|    | Glu   | Thr          | His | Gly | Thr | Lys | Asn | Tyr | Phe | Thr | Ser | Val | Ala | His | Pro |  |
|    |       |              |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| 10 | Asn   | Leu          | Phe | Ile | Ala | Thr | Lys | Gln | Asp | Tyr | Trp | Val | Cys | Leu | Ala |  |
|    |       |              |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |  |
|    | Gly   | Gly          | Pro | Pro | Ser | Ile | Thr | Asp | Phe | Gln | Ile | Leu | Glu | Asn | Gln |  |
|    |       |              |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |  |
| 15 | Ala   |              |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | 271   |              |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <210> | 26           |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 20 | <211> | 177          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <212> | PRT          |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <213> | Homo sapiens |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|    | <400> | 26           |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
| 25 | Met   | Glu          | Ile | Cys | Arg | Gly | Leu | Arg | Ser | His | Leu | Ile | Thr | Leu | Leu |  |
|    | 1     |              |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |  |
|    | Leu   | Phe          | Leu | Phe | His | Ser | Glu | Thr | Ile | Cys | Arg | Pro | Ser | Gly | Arg |  |
|    |       |              |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |  |
| 30 | Lys   | Ser          | Ser | Lys | Met | Gln | Ala | Phe | Arg | Ile | Trp | Asp | Val | Asn | Gln |  |
|    |       |              |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |  |
|    | Lys   | Thr          | Phe | Tyr | Leu | Arg | Asn | Asn | Gln | Leu | Val | Ala | Gly | Tyr | Leu |  |
| 35 |       |              |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |  |
|    | Gln   | Gly          | Pro | Asn | Val | Asn | Leu | Glu | Glu | Lys | Ile | Asp | Val | Val | Pro |  |
|    |       |              |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |  |
| 40 | Ile   | Glu          | Pro | His | Ala | Leu | Phe | Leu | Gly | Ile | His | Gly | Gly | Lys | Met |  |
|    |       |              |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |  |
|    | Cys   | Leu          | Ser | Cys | Val | Lys | Ser | Gly | Asp | Glu | Thr | Arg | Leu | Gln | Leu |  |
|    |       |              |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
| 45 | Glu   | Ala          | Val | Asn | Ile | Thr | Asp | Leu | Ser | Glu | Asn | Arg | Lys | Gln | Asp |  |
|    |       |              |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |



|    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|    | Lys | Arg | Phe | Ala | Phe | Ile | Arg | Ser | Asp | Ser | Gly | Pro | Thr | Thr | Ser |
|    |     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |
| 5  | Phe | Glu | Ser | Ala | Ala | Cys | Pro | Gly | Trp | Phe | Leu | Cys | Thr | Ala | Met |
|    |     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |
|    | Glu | Ala | Asp | Gln | Pro | Val | Ser | Leu | Thr | Asn | Met | Pro | Asp | Glu | Gly |
|    |     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |
| 10 | Val | Met | Val | Thr | Leu | Phe | Tyr | Phe | Gln | Glu | Asp | Glu |     |     |     |
|    |     |     |     |     | 170 |     |     |     |     | 175 |     | 177 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| Met | Asp | Trp | Pro | His | Asn | Leu | Leu | Phe | Leu | Leu | Thr | Ile | Ser | Ile | 1   | 5   | 10  | 15 |
| Phe | Leu | Gly | Leu | Gly | Gln | Pro | Arg | Ser | Pro | Lys | Ser | Lys | Arg | Lys | 20  | 25  | 30  |    |
| Gly | Gln | Gly | Arg | Pro | Gly | Pro | Leu | Ala | Pro | Gly | Pro | His | Gln | Val | 35  | 40  | 45  |    |
| Pro | Leu | Asp | Leu | Val | Ser | Arg | Met | Lys | Pro | Tyr | Ala | Arg | Met | Glu | 50  | 55  | 60  |    |
| Glu | Tyr | Glu | Arg | Asn | Ile | Glu | Glu | Met | Val | Ala | Gln | Leu | Arg | Asn | 65  | 70  | 75  |    |
| Ser | Ser | Glu | Leu | Ala | Gln | Arg | Lys | Cys | Glu | Val | Asn | Leu | Gln | Leu | 80  | 85  | 90  |    |
| Trp | Met | Ser | Asn | Lys | Arg | Ser | Leu | Ser | Pro | Trp | Gly | Tyr | Ser | Ile | 95  | 100 | 105 |    |
| Asn | His | Asp | Pro | Ser | Arg | Ile | Pro | Val | Asp | Leu | Pro | Glu | Ala | Arg | 110 | 115 | 120 |    |
| Cys | Leu | Cys | Leu | Gly | Cys | Val | Asn | Pro | Phe | Thr | Met | Gln | Glu | Asp | 125 | 130 | 135 |    |
| Arg | Ser | Met | Val | Ser | Val | Pro | Val | Phe | Ser | Gln | Val | Pro | Val | Arg | 140 | 145 | 150 |    |
| Arg | Arg | Leu | Cys | Pro | Pro | Pro | Pro | Arg | Thr | Gly | Pro | Cys | Arg | Gln | 155 | 160 | 165 |    |
| Arg | Ala | Val | Met | Glu | Thr | Ile | Ala | Val | Gly | Cys | Thr | Cys | Ile | Phe | 170 | 175 | 180 |    |

FIGURE 1

aggcgggcag cagctgcagg ctgacctgac agcttggcgg aatggactgg 50  
cctcacaacc tgctgtttct tcttaccatt tccatcttcc tggggctggg 100  
ccagcccagg agccccaaaa gcaagaggaa ggggcaaggg cggcctgggc 150  
ccctggcccc tggccctcac caggtgccac tggacctggt gtcacggatg 200  
aaaccgtatg cccgcatgga ggagtatgag aggaacatcg aggagatggt 250  
ggcccagctg aggaacagct cagagctggc ccagagaaaag tgtgaggtca 300  
acttgcagct gtggatgtcc aacaagagga gcctgtctcc ctggggctac 350  
agcatcaacc acgaccccag ccgtatcccc gtggacctgc cggaggcacg 400  
gtgcctgtgt ctgggctgtg tgaaccctt caccatgcag gaggaccgca 450  
gcatggtgag cgtgccggtg ttcagccagg ttcctgtgcg ccgccgcctc 500  
tgcccgccac cgccccgcac agggccttgc cgccagcgcg cagtcatgga 550  
gaccatcgct gtgggctgca cctgcatctt ctgaatcacc tggcccagaa 600  
gccaggccag cagcccgaga ccatactcct tgcacctttg tgccaagaaa 650  
ggcctatgaa aagtaaacac tgacttttga aagcaag 687

FIGURE 2

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |    |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|
| Met | Thr | Leu | Leu | Pro | Gly | Leu | Leu | Phe | Leu | Thr | Trp | Leu | His | Thr | 1   | 5   | 10  | 15 |
| Cys | Leu | Ala | His | His | Asp | Pro | Ser | Leu | Arg | Gly | His | Pro | His | Ser | 20  | 25  | 30  |    |
| His | Gly | Thr | Pro | His | Cys | Tyr | Ser | Ala | Glu | Glu | Leu | Pro | Leu | Gly | 35  | 40  | 45  |    |
| Gln | Ala | Pro | Pro | His | Leu | Leu | Ala | Arg | Gly | Ala | Lys | Trp | Gly | Gln | 50  | 55  | 60  |    |
| Ala | Leu | Pro | Val | Ala | Leu | Val | Ser | Ser | Leu | Glu | Ala | Ala | Ser | His | 65  | 70  | 75  |    |
| Arg | Gly | Arg | His | Glu | Arg | Pro | Ser | Ala | Thr | Thr | Gln | Cys | Pro | Val | 80  | 85  | 90  |    |
| Leu | Arg | Pro | Glu | Glu | Val | Leu | Glu | Ala | Asp | Thr | His | Gln | Arg | Ser | 95  | 100 | 105 |    |
| Ile | Ser | Pro | Trp | Arg | Tyr | Arg | Val | Asp | Thr | Asp | Glu | Asp | Arg | Tyr | 110 | 115 | 120 |    |
| Pro | Gln | Lys | Leu | Ala | Phe | Ala | Glu | Cys | Leu | Cys | Arg | Gly | Cys | Ile | 125 | 130 | 135 |    |
| Asp | Ala | Arg | Thr | Gly | Arg | Glu | Thr | Ala | Ala | Leu | Asn | Ser | Val | Arg | 140 | 145 | 150 |    |
| Leu | Leu | Gln | Ser | Leu | Leu | Val | Leu | Arg | Arg | Arg | Pro | Cys | Ser | Arg | 155 | 160 | 165 |    |
| Asp | Gly | Ser | Gly | Leu | Pro | Thr | Pro | Gly | Ala | Phe | Ala | Phe | His | Thr | 170 | 175 | 180 |    |
| Glu | Phe | Ile | His | Val | Pro | Val | Gly | Cys | Thr | Cys | Val | Leu | Pro | Arg | 185 | 190 | 195 |    |
| Ser | Val |     |     |     |     |     |     |     |     |     |     |     |     |     | 197 |     |     |    |

FIGURE 3

gccaggtgtg caggccgctc caagcccagc ctgccccgct gccgccacca 50  
tgacgctcct ccccggcctc ctgtttctga cctggctgca cacatgcctg 100  
gcccaccatg acccctccct cagggggcac ccccacagtc acggtacccc 150  
aactgctac tcggctgagg aactgcccct cggccaggcc cccccacacc 200  
tgctggctcg aggtgccaaag tgggggcagg ctttgccctgt agccctgggtg 250  
tccagcctgg aggcagcaag ccacaggggg aggcacgaga ggccctcagc 300  
tacgaccag tgcccgggtgc tgcggccgga ggaggtgttg gaggcagaca 350  
cccaccagcg ctccatctca ccctggagat accgtgtgga cacggatgag 400  
gaccgctatc cacagaagct ggccttcgcc gagtgcctgt gcagaggctg 450  
tatcgatgca cggacgggcc gcgagacagc tgcgtcaac tccgtgcggc 500  
tgctccagag cctgctgggtg ctgcgcgcc ggccctgctc ccgcgacggc 550  
tcggggctcc ccacacctgg ggcctttgcc ttccacaccg agttcatcca 600  
cgtccccgtc ggctgcacct gcgtgctgcc ccgttcagtg tgaccgccga 650  
ggccgtgggg cccctagact ggacacgtgt gctccccaga gggcaccccc 700  
tatttatgtg tatttattgt tatttatatg cttccccaa cactaccctt 750  
ggggtctggg cattccccgt gtctggagga cagccccca ctgttctcct 800  
catctccagc ctcagtagtt gggggtagaa ggagctcagc acctcttcca 850  
gcccttaaag ctgcagaaaa ggtgtcacac ggctgcctgt acctggctc 900  
cctgtcctgc tcccggcttc ccttacccta tctaggcct caggccccgc 950  
aggctgcctc ttcccaacct ccttgaagt acccctgttt cttaacaat 1000  
tatttaagtg tacgtgtatt attaaactga tgaacacatc cccaaaa 1047

FIGURE 4

ggcagcaggg accaagagag gcacgcttgc ccttttatga catcagagct 50  
 cctgggttctt gctccttggg actctgggac ttacaccagt ggcaccctg 100  
 gctcnnnnnn nnnnnaattc ggtacgaggc tgggggttcag gcgggcagca 150  
 gctgcaggct gaccttgcag cttggcgga tggactggcc tcacaacctg 200  
 ctgtttcttc ttaccatttc catcttctg gggctgggcc agcccaggag 250  
 ccccaaaagc aagaggaagg ggcaaggcg gcctgggccc ctggtcctg 300  
 gccctcacca ggtgccactg gacctggtgt cacggatgaa accgtatgcc 350  
 cgcatggagg agtatgagag gaacatcgag gagatgttgg ccagctgag 400  
 gaacagttca gagctggccc agagaaagtg tgaggtaaac ttgcagctgt 450  
 ggatgtccaa caagaggagc ctgtctcctt ggggctacag catcaaccac 500  
 gaccccagcc gtatccccgt ggacctcgg aggcacggtg cctgtgtctg 550  
 ggcttgtgtg aacccttca ccatgcagga ggaccgcagc atggtgagcg 600  
 tgccggtgtt cagccagggt cctgtgcgcc gccgcctctg cccgccaccg 650  
 ccccgcacag ggccttgccg ccagcgcgca gtcattggaga ccatcgctgt 700  
 gggctgcacc tgcattctct gaatcgacct ggcccagaag ccaggccagc 750  
 agcccagagac catctcctt gcacctttgt gccaaagaa gcctatgaaa 800  
 agtaaact gacttttgaa agcaaaaaaa 830

FIGURE 5

cacggatgag gaccgctatc cacagaagct ggccttcgcc gagtgcctgt 50  
gcagaggctg tatcgatgca cggacggggcc gcgagacagc tgcgctcaac 100  
tccgtgcggc tgctccagag cctgctggtg ctgcgccgcc ggccttgctc 150  
ccgcgacggc tcggggctcc ccacacctgg ggcctttgcc ttccacaccg 200  
agttcatcca cgtccccgtc ggctgcacct 230

FIGURE 6

|        |     |   |
|--------|-----|---|
| HIL17  | 1   | .....MTPGKISLVSLLL  |
| HIL17B | 1   | .....MDWPHNLLFLTLLTISIFLGLGQPRSPKSKRKGGGRPGPLAPGP   |
| HIL17C | 1   | MTLLPGLLFLTWLHTCLAHHDPSLRGHPHSHGTPHCYSAEELPLGQAPPH  |
| HIL17  | 16  | SL <sup>1</sup> EAIV <sup>2</sup> KA <sup>3</sup> GI <sup>4</sup> TI <sup>5</sup> PRNPGCPNSEDKNFP <sup>6</sup> RTVMV <sup>7</sup> NLNINHRNTNTPKRSS <sup>8</sup> D   |
| HIL17B | 43  | HOVPLDLVSRMK <sup>1</sup> PYA <sup>2</sup> RMEEYE <sup>3</sup> ERNIEEMVAQLR <sup>4</sup> MSSELAQRKCEVNLQWM  |
| HIL17C | 51  | L <sup>1</sup> ARGAK <sup>2</sup> W <sup>3</sup> QAL <sup>4</sup> PVALVSSLEAASHRG <sup>5</sup> RHERPSATTQCPLRPEEVLEAD   |
| HIL17  | 66  | YYN <sup>1</sup> RS <sup>2</sup> TS <sup>3</sup> SPWNLH <sup>4</sup> RNE <sup>5</sup> D <sup>6</sup> PERYP <sup>7</sup> SVIWEAK <sup>8</sup> CRHL <sup>9</sup> GC <sup>10</sup> LNADGNVDYHMSVPI   |
| HIL17B | 93  | SNK <sup>1</sup> RS <sup>2</sup> LS <sup>3</sup> SPWGY <sup>4</sup> SI <sup>5</sup> NH <sup>6</sup> DP <sup>7</sup> SR <sup>8</sup> IP <sup>9</sup> VDL <sup>10</sup> PEARCL <sup>11</sup> CGCV <sup>12</sup> W <sup>13</sup> PF <sup>14</sup> TMOED <sup>15</sup> DRSMV <sup>16</sup> SV <sup>17</sup> P |
| HIL17C | 101 | THQRS <sup>1</sup> IS <sup>2</sup> SPWRY <sup>3</sup> RVDT <sup>4</sup> DEDRYP <sup>5</sup> OKLA <sup>6</sup> FAECL <sup>7</sup> CRG <sup>8</sup> CLD <sup>9</sup> ART <sup>10</sup> GRE <sup>11</sup> TAA <sup>12</sup> LN <sup>13</sup> SV <sup>14</sup> R  |
| HIL17  | 116 | QOEI <sup>1</sup> .LVLRRE <sup>2</sup> PH <sup>3</sup> CRNS <sup>4</sup> .....ERLE <sup>5</sup> K <sup>6</sup> LL <sup>7</sup> SVGCTCV <sup>8</sup> TI <sup>9</sup> IVHVA   |
| HIL17B | 143 | VFSQVP <sup>1</sup> VR <sup>2</sup> RL <sup>3</sup> ...CBPP <sup>4</sup> PRT <sup>5</sup> ...GPCRO <sup>6</sup> RAV <sup>7</sup> METIA <sup>8</sup> VGCT <sup>9</sup> dlf <sup>10</sup> .....   |
| HIL17C | 151 | LLQSL <sup>1</sup> LVLRBR <sup>2</sup> RP <sup>3</sup> ...CSR <sup>4</sup> DGSG <sup>5</sup> LP <sup>6</sup> T <sup>7</sup> PGAFA <sup>8</sup> EH <sup>9</sup> TE <sup>10</sup> FL <sup>11</sup> HM <sup>12</sup> PVGCIC <sup>13</sup> VL <sup>14</sup> BR <sup>15</sup> SV...                            |

FIGURE 7A



Tue Apr 27 16:58:30 1999

/home/ruby/va/Molbio/carpanda/temp/aa.out

```
59294 1 M D W P H N L L F L L T I S I F L G L G Q P R S P K S K R K G Q G R P G P L A P G P . . . H Q V P L
62377 1 M T L L P G L L F L T W L H T C L A H H D P . S L R G H P H S H G T P H C Y S A E E L P L G Q A P P

59294 48 D L V S R M K P Y A R M . . E E Y E R N I E E M V A Q L R N S S E L A Q R K C E V . . . N L Q L W
62377 50 H L L A R G A K W G O A L P V A L V S S L E A A S H R G R H E R P S A T T Q C P V L R P E E V L E A

59294 92 M S N K R S L S P W G Y S I N H D P S R I P V D L P E A R C L C L G C V N P F T M Q E D R S M V S V
62377 100 D T H Q R S I S P W R Y R V D T D E D R Y P Q K L A F A E C L C R G C I D A R T G R E T A A L N S V

59294 142 P V F . S Q V P V R R R L C P P P . . . P R T G P C R Q R A V M E T I A V G C T C I F
62377 150 R L L Q S L L V L R R R P C S R D G S G L P T P G A F A F H T E F I H V P V G C T C V L P R S V
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FIGURE 7B

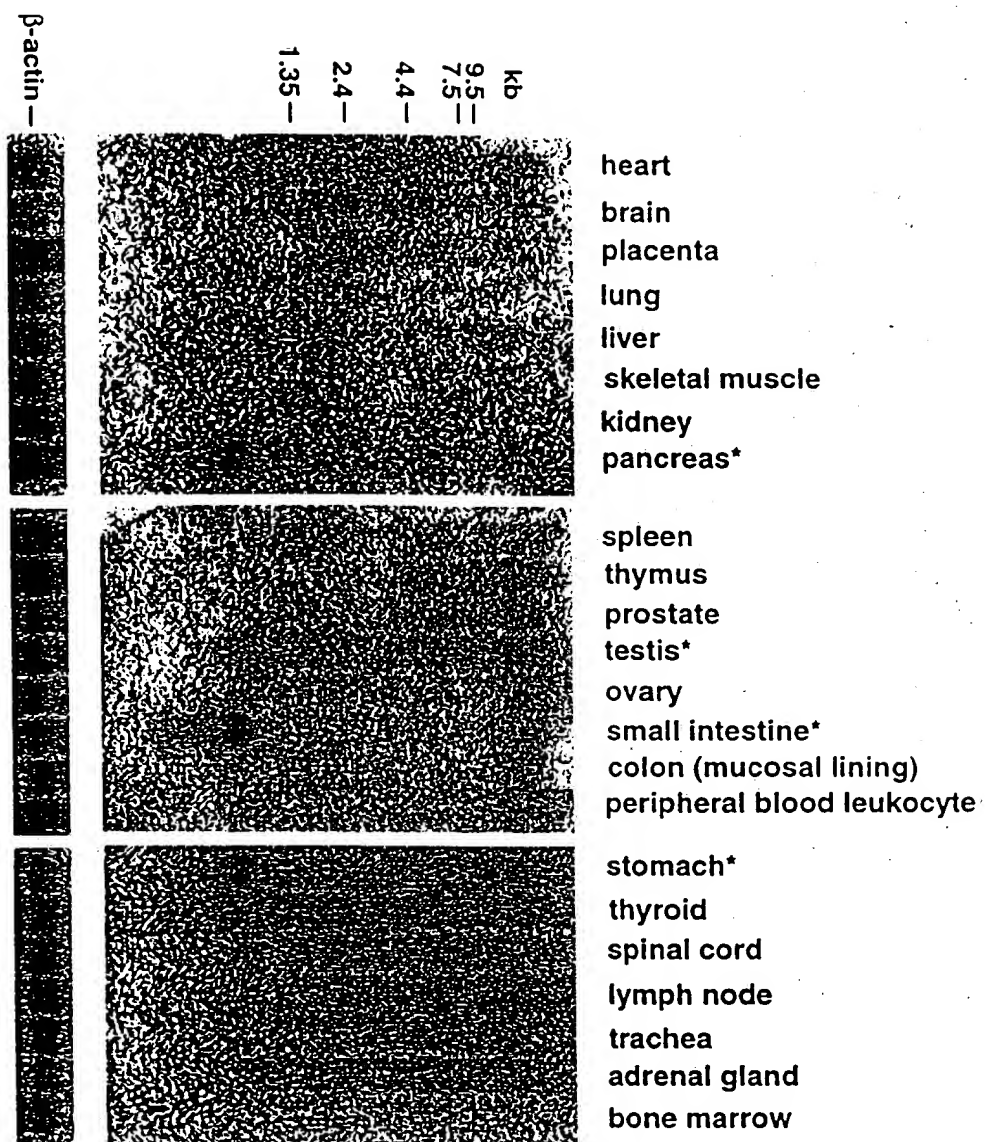
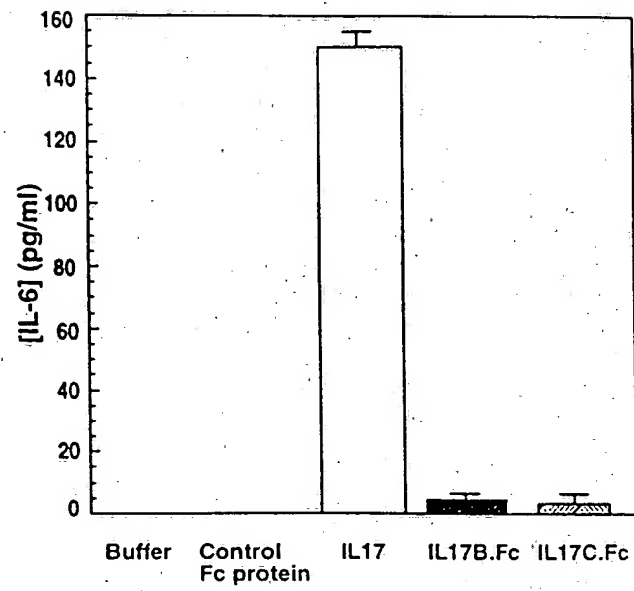
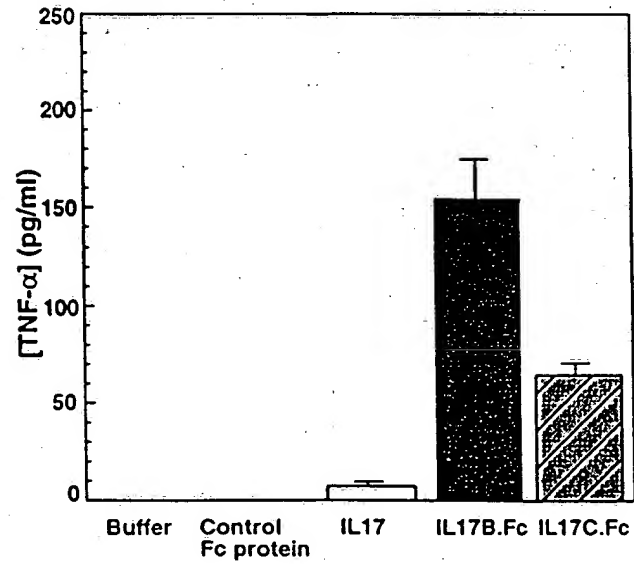


FIGURE 8

**A. HFF cells**

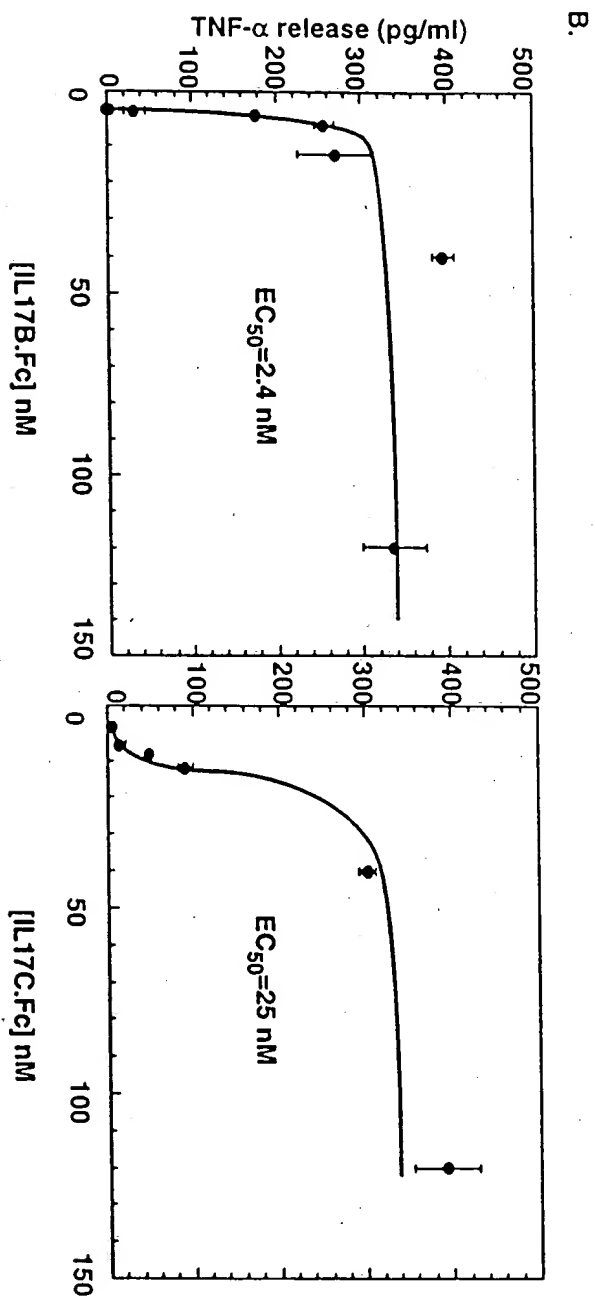
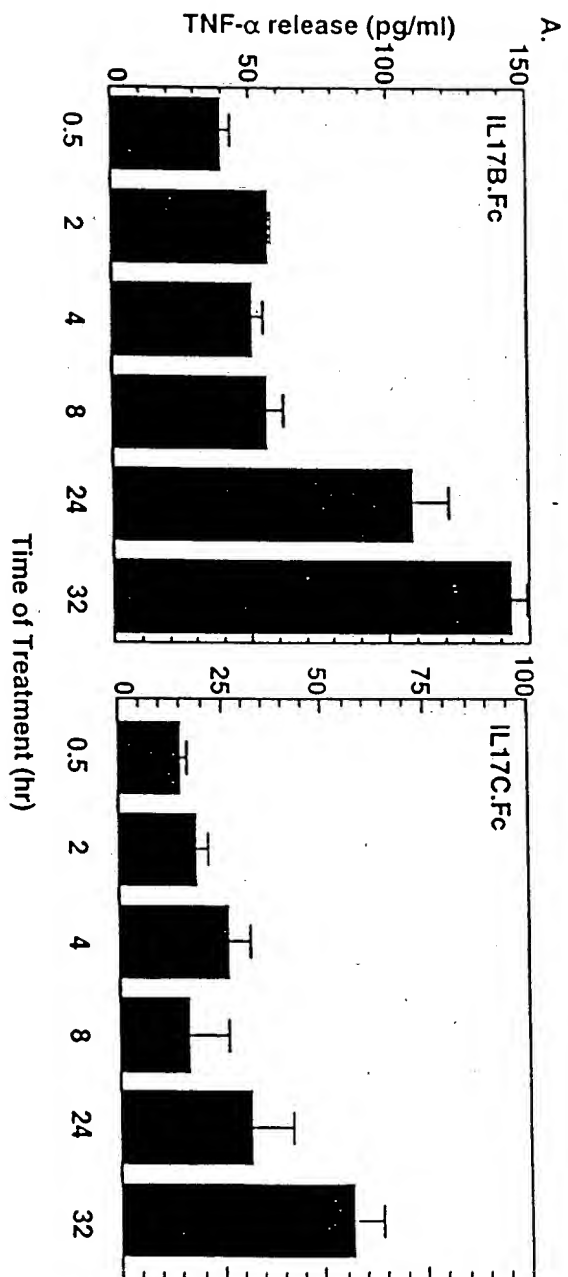


**B. THP1 cells**



**FIGURE 9**

FIGURE 10



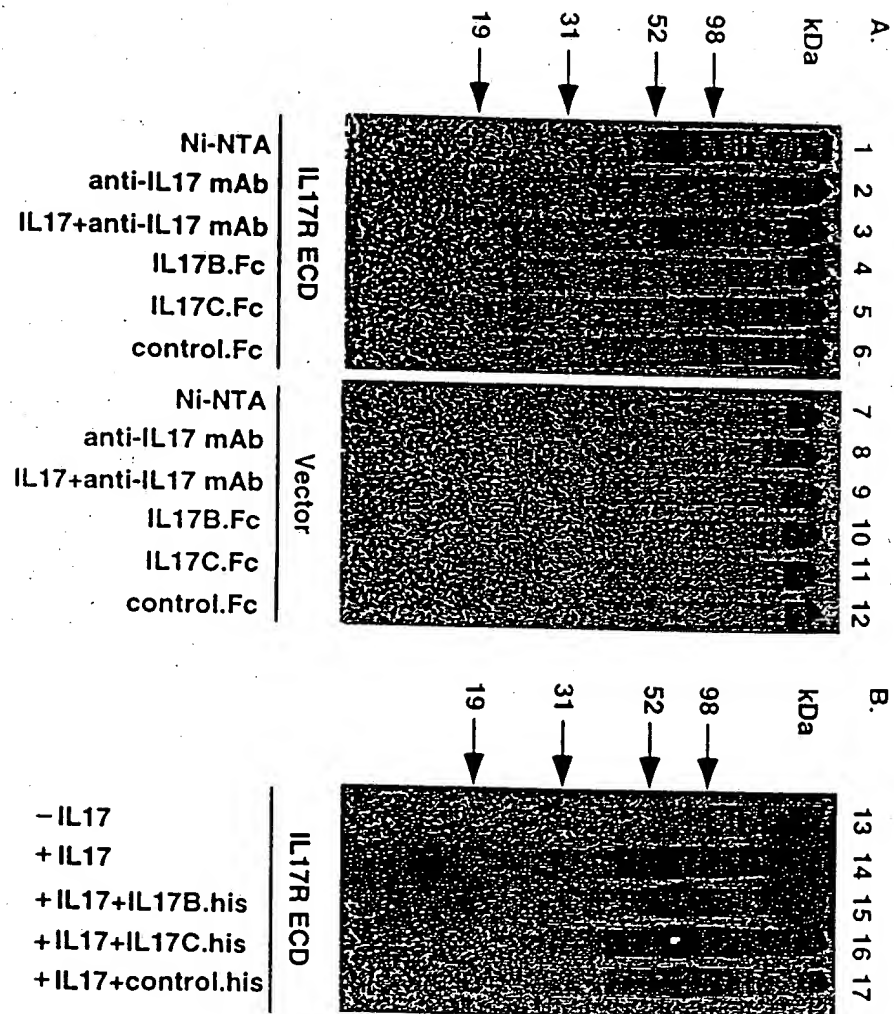
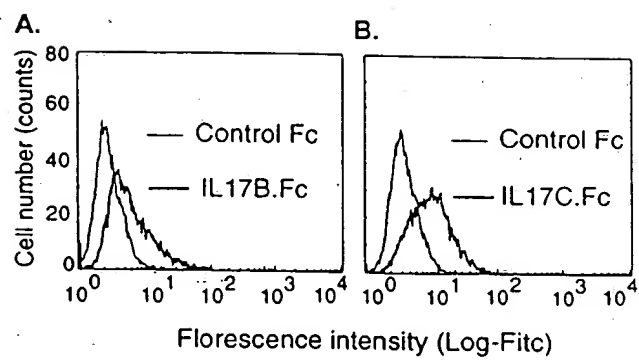


FIGURE 11



**FIGURE 12**

# IL-17 induces breakdown and inhibits synthesis of cartilage matrix

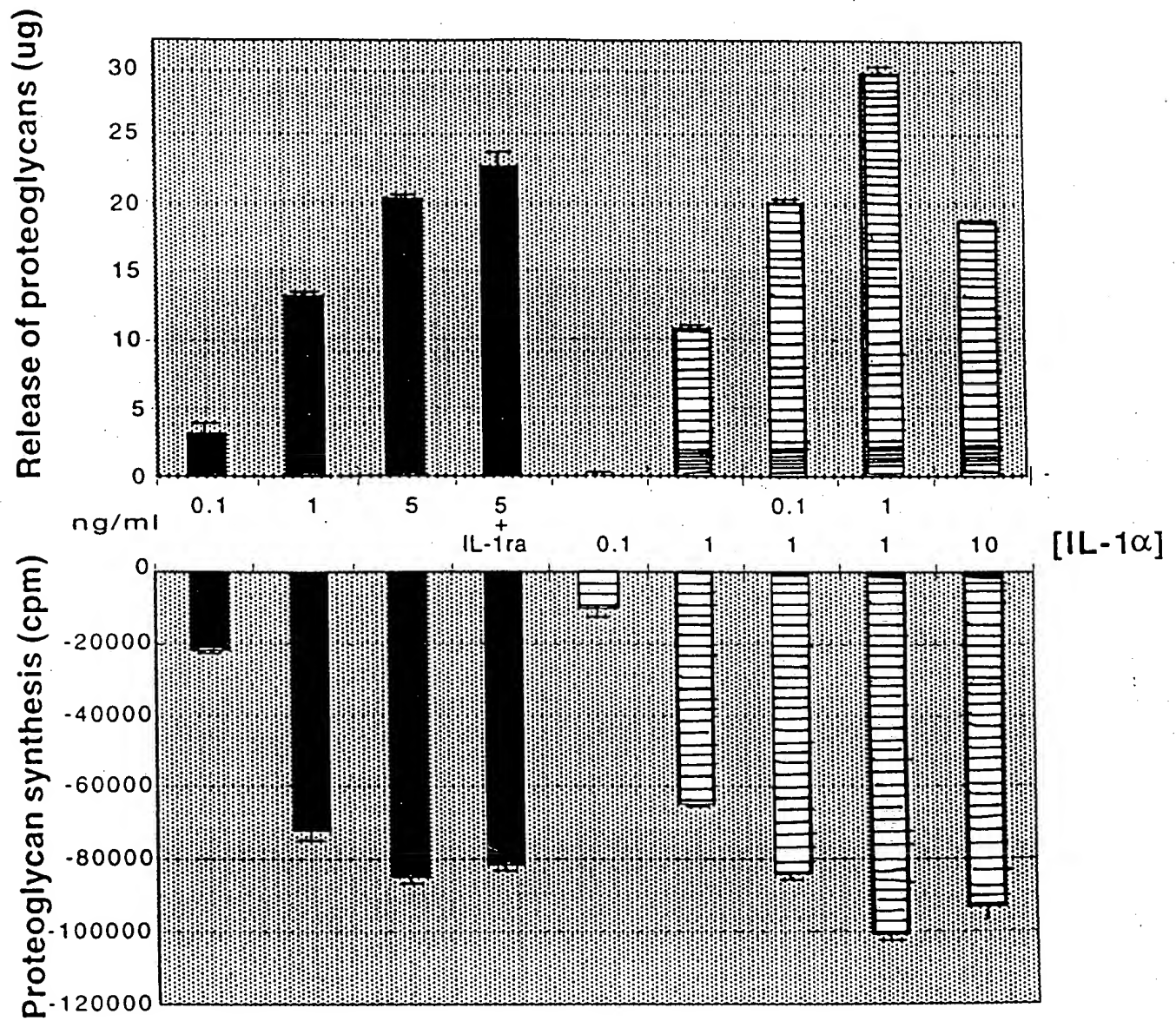
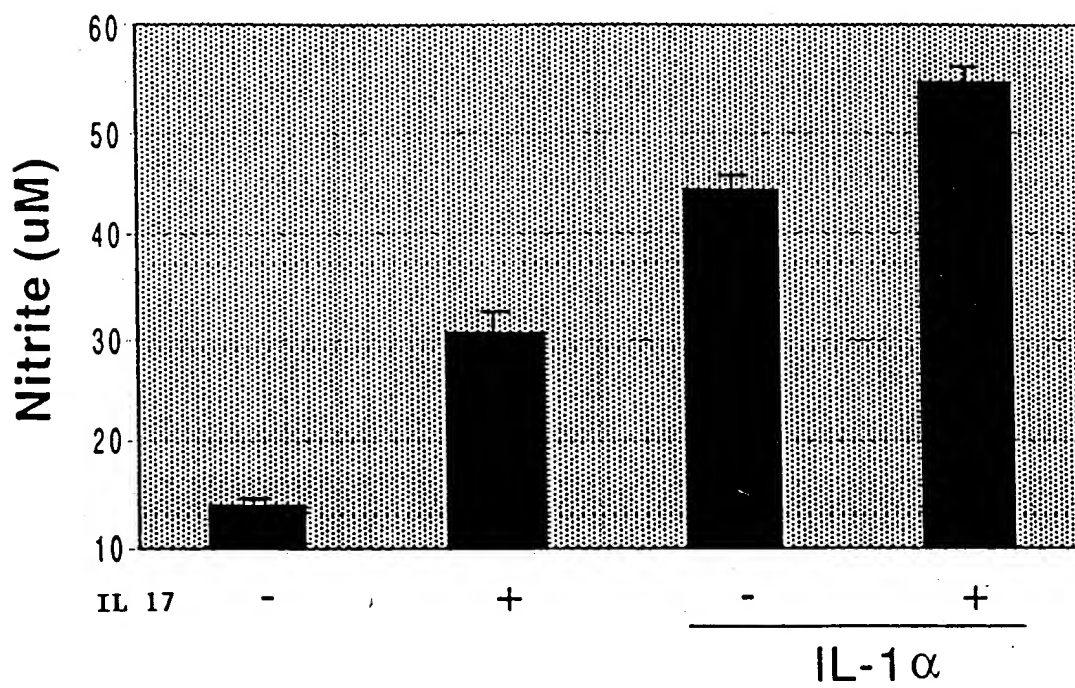


FIGURE 13

**IL 17 increases basal and  
IL-1 $\alpha$ -induced nitric oxide release**

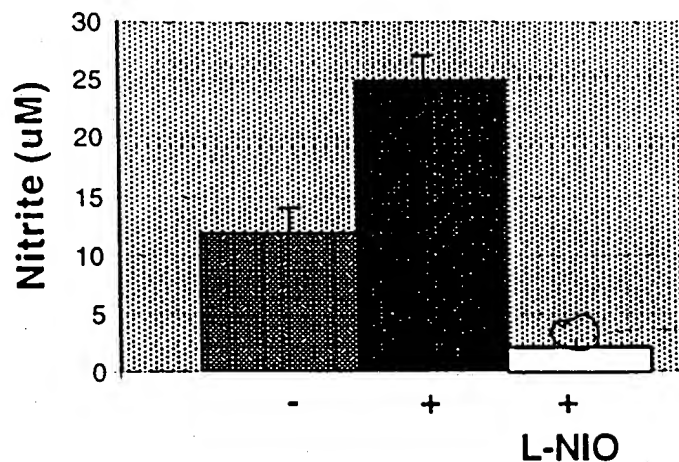


**FIGURE 14**

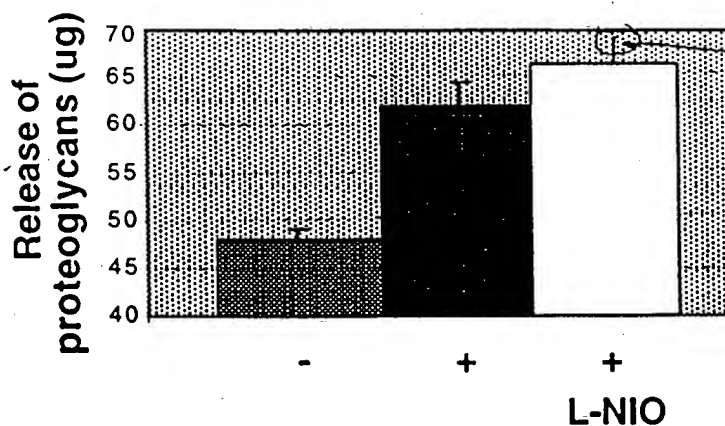


# Inhibition of nitric oxide release does not block the detrimental effects of IL 17 on matrix breakdown or synthesis

A.



B.



C.

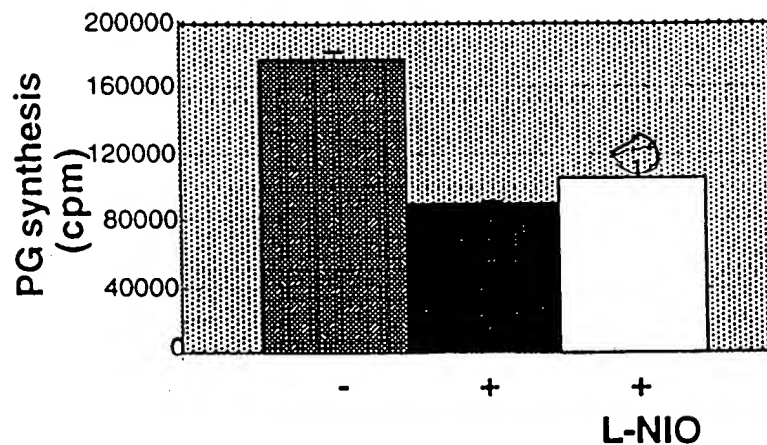


FIGURE 15

INHIBITION of NO release enhances  
 IL-1- $\alpha$ -induced matrix breakdown  
 but not matrix synthesis

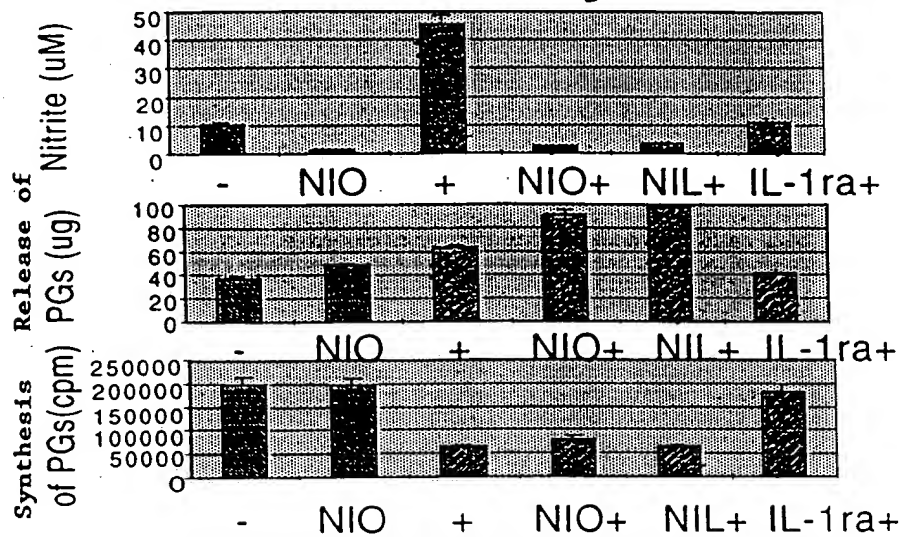


FIGURE 16

IL 17 homologue 1 (UNQ516)  
has positive effects on  
articular cartilage

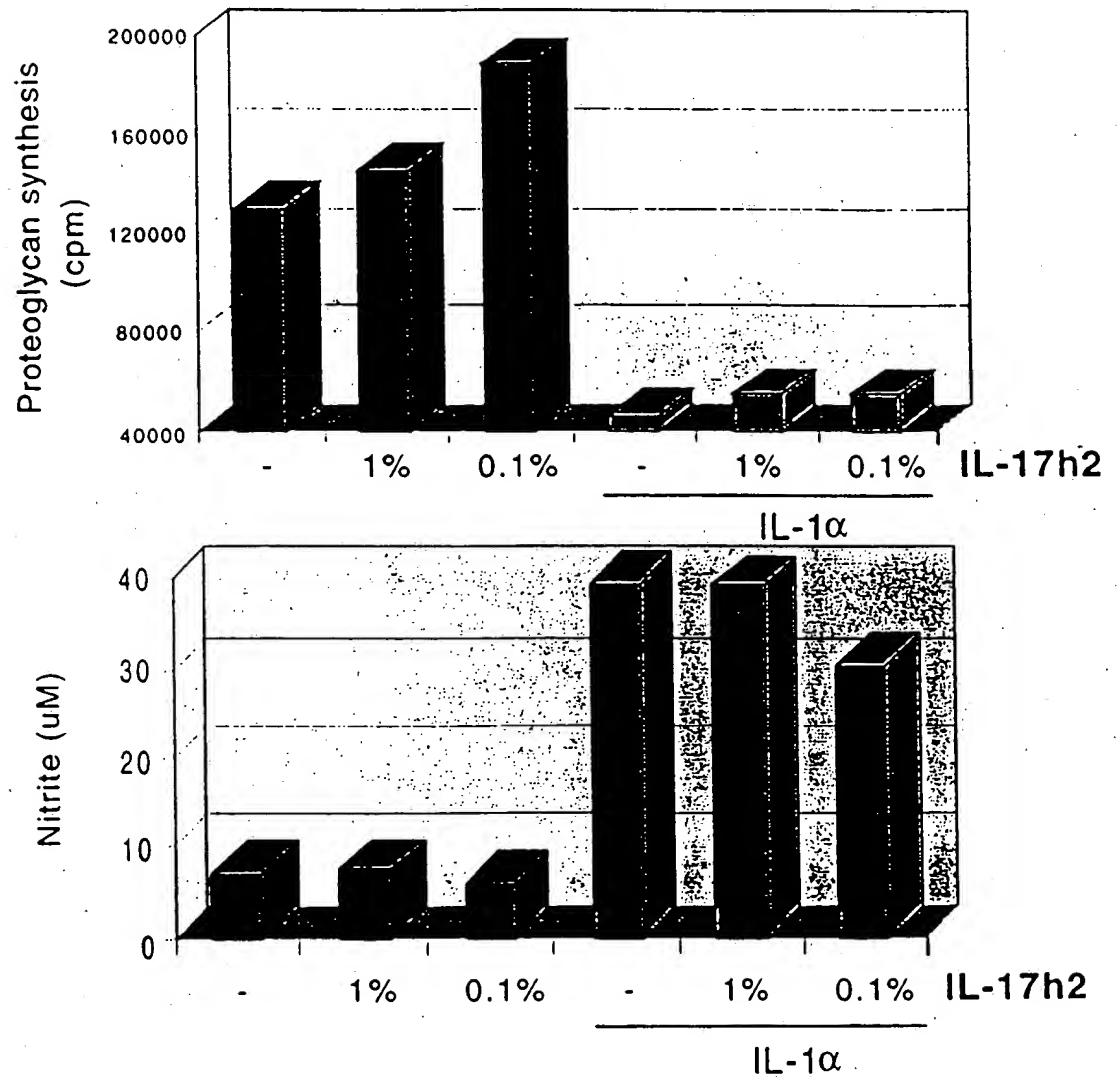


FIGURE 17

# IL 17 homologue (UNQ 561) has detrimental effects on articular cartilage

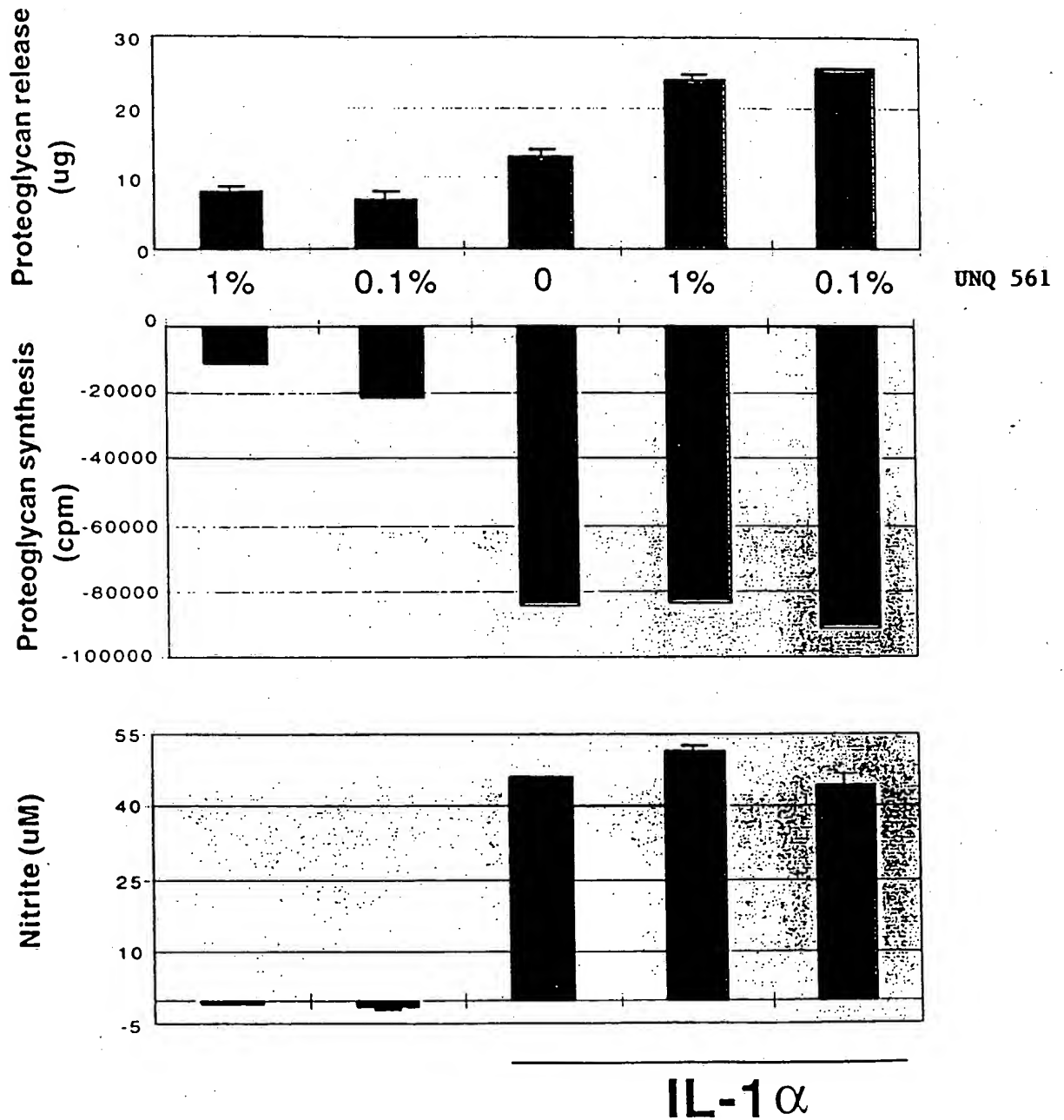


FIGURE 18

Sequence Listing

<110> Chen, Jian  
 Filvaroff, Ellen  
 Goddard, Audrey  
 Gurney, Austin  
 Li, Hanzhong  
 Wood, William I.

<120> IL-17 HOMOLOGOUS POLYPEPTIDES AND THERAPEUTIC USES THEREOF

<130> P1381-R1

<141> 1999-05-14

<150> US 60/085,579

<151> 1998-05-15

<150> US 60/113,621

<151> 1998-12-23

<160> 26

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<211> 180

<212> PRT

<213> Homo sapiens

<400> 1

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asp | Trp | Pro | His | Asn | Leu | Leu | Phe | Leu | Leu | Thr | Ile | Ser | Ile |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Leu | Gly | Leu | Gly | Gln | Pro | Arg | Ser | Pro | Lys | Ser | Lys | Arg | Lys |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gln | Gly | Arg | Pro | Gly | Pro | Leu | Ala | Pro | Gly | Pro | His | Gln | Val |
|     |     |     | 35  |     |     |     |     | 40  |     |     |     |     |     | 45  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Asp | Leu | Val | Ser | Arg | Met | Lys | Pro | Tyr | Ala | Arg | Met | Glu |
|     |     |     | 50  |     |     |     |     | 55  |     |     |     |     |     | 60  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Tyr | Glu | Arg | Asn | Ile | Glu | Glu | Met | Val | Ala | Gln | Leu | Arg | Asn |
|     |     |     | 65  |     |     |     |     | 70  |     |     |     |     |     | 75  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ser | Glu | Leu | Ala | Gln | Arg | Lys | Cys | Glu | Val | Asn | Leu | Gln | Leu |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Trp | Met | Ser | Asn | Lys | Arg | Ser | Leu | Ser | Pro | Trp | Gly | Tyr | Ser | Ile |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | His | Asp | Pro | Ser | Arg | Ile | Pro | Val | Asp | Leu | Pro | Glu | Ala | Arg |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Leu | Cys | Leu | Gly | Cys | Val | Asn | Pro | Phe | Thr | Met | Gln | Glu | Asp |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Ser | Met | Val | Ser | Val | Pro | Val | Phe | Ser | Gln | Val | Pro | Val | Arg |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Arg | Leu | Cys | Pro | Pro | Pro | Pro | Arg | Thr | Gly | Pro | Cys | Arg | Gln |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Ala | Val | Met | Glu | Thr | Ile | Ala | Val | Gly | Cys | Thr | Cys | Ile | Phe |
|     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |

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&lt;211&gt; 687

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

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ggcccagctg aggaacagct cagagctggc ccagagaaag tgtgaggtca 300
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agcatcaacc acgaccccag ccgtatcccc gtggacctgc cggaggcacg 400

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<213> Homo sapiens

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Leu | Leu | Pro | Gly | Leu | Leu | Phe | Leu | Thr | Trp | Leu | His | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
| Cys | Leu | Ala | His | His | Asp | Pro | Ser | Leu | Arg | Gly | His | Pro | His | Ser |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |
| His | Gly | Thr | Pro | His | Cys | Tyr | Ser | Ala | Glu | Glu | Leu | Pro | Leu | Gly |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |
| Gln | Ala | Pro | Pro | His | Leu | Leu | Ala | Arg | Gly | Ala | Lys | Trp | Gly | Gln |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |
| Ala | Leu | Pro | Val | Ala | Leu | Val | Ser | Ser | Leu | Glu | Ala | Ala | Ser | His |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |
| Arg | Gly | Arg | His | Glu | Arg | Pro | Ser | Ala | Thr | Thr | Gln | Cys | Pro | Val |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |
| Leu | Arg | Pro | Glu | Glu | Val | Leu | Glu | Ala | Asp | Thr | His | Gln | Arg | Ser |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |
| Ile | Ser | Pro | Trp | Arg | Tyr | Arg | Val | Asp | Thr | Asp | Glu | Asp | Arg | Tyr |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |

Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile  
125 130 135

Asp Ala Arg Thr Gly Arg Glu Thr Ala Ala Leu Asn Ser Val Arg  
140 145 150

Leu Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg  
155 160 165

Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr  
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Glu Phe Ile His Val Pro Val Gly Cys Thr Cys Val Leu Pro Arg  
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Ser Val  
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gccaccatg acccctcctt cagggggcac cccacagtc acggtacccc 150

acactgctac tcggctgagg aactgccctt cggccaggcc cccccacacc 200

tgctggctcg aggtgccaag tgggggcagg ctttgctgt agccctgggtg 250

tccagcctgg aggcagcaag ccacaggggg aggcacgaga ggccctcagc 300

tacgaccag tgcccggtgc tgcggccgga ggaggtgttg gaggcagaca 350

cccaccagcg ctccatctca ccttgagat accgtgtgga cacggatgag 400

gaccgctatc cacagaagct ggccttcgcc gaggctgtg gcagaggctg 450

tatcgatgca cggacgggccc gcgagacagc tgcgctcaac tccgtgcggc 500



tgctccagag cctgctggtg ctgcgccgcc ggccctgctc ccgcgacggc 550  
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 gctcnnnnnn nnnnaattc ggtacgaggc tggggttcag gcgggcagca 150  
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 ctgtttcttc ttaccatttc catcttctg gggctgggccc agcccaggag 250

ccccaaaagc aagaggaagg ggcaagggcg gcctggggccc ctggtccctg 300  
 gccctcacca ggtgccactg gacctggtgt cacggatgaa accgtatgcc 350  
 cgcattggagg agtatgagag gaacatcgag gagatggttg cccagctgag 400  
 gaacagttca gagctggccc agagaaagtg tgaggtcaac ttgcagctgt 450  
 ggatgtccaa caagaggagc ctgtctccct ggggctacag catcaaccac 500  
 gaccccagcc gtatccccgt ggacctccgg aggcacggtg cctgtgtctg 550  
 ggcttgtgtg aacccttca ccatgcagga ggaccgcagc atggtgagcg 600  
 tgccggtgtt cagccagggt cctgtgcgcc gccgcctctg cccgccaccg 650  
 ccccgcacag ggccttgccg ccagcgcgca gtcattggaga ccatcgctgt 700  
 gggctgcacc tgcattctct gaatcgacct gggccagaag ccaggccagc 750  
 agcccgagac catctctctt gcacctttgt gccaaagaaag gcctatgaaa 800  
 agtaaact gacttttgaa agcaaaaaaa 830

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 agccaggagc cccaaaagca agaggaaggg gcaagggcgg cctggggccn 150  
 tggcctggcc tcaccagggt cactggacc tgggtgtcac gatgaaaccg 200

tatgcccgcga tggaggagta tgagaggaac atcgaggaga tgggtggccca 250  
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cagctgtgga tgtccaacaa gaaggagcct gtctcccttg gggctacaag 350  
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tccgtgcggc tgctccagag cctgctggtg ctgcgccgcc ggccttgctc 150  
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&lt;213&gt; Artificial sequence

&lt;400&gt; 10

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&lt;210&gt; 11

&lt;211&gt; 155

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 11

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Pro | Gly | Lys | Thr | Ser | Leu | Val | Ser | Leu | Leu | Leu | Leu | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Leu | Glu | Ala | Ile | Val | Lys | Ala | Gly | Ile | Thr | Ile | Pro | Arg | Asn |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gly | Cys | Pro | Asn | Ser | Glu | Asp | Lys | Asn | Phe | Pro | Arg | Thr | Val |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Val | Asn | Leu | Asn | Ile | His | Asn | Arg | Asn | Thr | Asn | Thr | Asn | Pro |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Arg | Ser | Ser | Asp | Tyr | Tyr | Asn | Arg | Ser | Thr | Ser | Pro | Trp | Asn |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | His | Arg | Asn | Glu | Asp | Pro | Glu | Arg | Tyr | Pro | Ser | Val | Ile | Trp |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ala | Lys | Cys | Arg | His | Leu | Gly | Cys | Ile | Asn | Ala | Asp | Gly | Asn |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Asp | Tyr | His | Met | Asn | Ser | Val | Pro | Ile | Gln | Gln | Glu | Ile | Leu |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Leu | Arg | Arg | Glu | Pro | Pro | His | Cys | Pro | Asn | Ser | Phe | Arg | Leu |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Lys | Ile | Leu | Val | Ser | Val | Gly | Cys | Thr | Cys | Val | Thr | Pro | Ile |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |

Val His His Val Ala

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<211> 408

<212> PRT

<213> Artificial

<220>

<223> Artificial Sequence 1-408

<400> 12

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Asp | Trp | Pro | His | Asn | Leu | Leu | Phe | Leu | Leu | Thr | Ile | Ser | Ile |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Leu | Gly | Leu | Gly | Gln | Pro | Arg | Ser | Pro | Lys | Ser | Lys | Arg | Lys |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gln | Gly | Arg | Pro | Gly | Pro | Leu | Ala | Pro | Gly | Pro | His | Gln | Val |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Asp | Leu | Val | Ser | Arg | Met | Lys | Pro | Tyr | Ala | Arg | Met | Glu |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Tyr | Glu | Arg | Asn | Ile | Glu | Glu | Met | Val | Ala | Gln | Leu | Arg | Asn |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Ser | Glu | Leu | Ala | Gln | Arg | Lys | Cys | Glu | Val | Asn | Leu | Gln | Leu |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Met | Ser | Asn | Lys | Arg | Ser | Leu | Ser | Pro | Trp | Gly | Tyr | Ser | Ile |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | His | Asp | Pro | Ser | Arg | Ile | Pro | Val | Asp | Leu | Pro | Glu | Ala | Arg |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Leu | Cys | Leu | Gly | Cys | Val | Asn | Pro | Phe | Thr | Met | Gln | Glu | Asp |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ser | Met | Val | Ser | Val | Pro | Val | Phe | Ser | Gln | Val | Pro | Val | Arg |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Arg | Leu | Cys | Pro | Pro | Pro | Pro | Arg | Thr | Gly | Pro | Cys | Arg | Gln |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ala | Val | Met | Glu | Thr | Ile | Ala | Val | Gly | Cys | Thr | Cys | Ile | Phe | 170 | 175 | 180 |
| Pro | Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Leu | 185 | 190 | 195 |
| Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | 200 | 205 | 210 |
| Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | 215 | 220 | 225 |
| Asp | Val | Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | 230 | 235 | 240 |
| Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | 245 | 250 | 255 |
| Gln | Tyr | Asn | Ser | Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | 260 | 265 | 270 |
| His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | 275 | 280 | 285 |
| Asn | Lys | Ala | Leu | Pro | Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala | 290 | 295 | 300 |
| Lys | Gly | Gln | Pro | Arg | Glu | Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | 305 | 310 | 315 |
| Arg | Glu | Glu | Met | Thr | Lys | Asn | Gln | Val | Ser | Leu | Thr | Cys | Leu | Val | 320 | 325 | 330 |
| Lys | Gly | Phe | Tyr | Pro | Ser | Asp | Ile | Ala | Val | Glu | Trp | Glu | Ser | Asn | 335 | 340 | 345 |
| Gly | Gln | Pro | Glu | Asn | Asn | Tyr | Lys | Thr | Thr | Pro | Pro | Val | Leu | Asp | 350 | 355 | 360 |
| Ser | Asp | Gly | Ser | Phe | Phe | Leu | Tyr | Ser | Lys | Leu | Thr | Val | Asp | Lys | 365 | 370 | 375 |

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
380 385 390

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
395 400 405

Pro Gly Lys  
408

<210> 13

<211> 425

<212> PRT

<213> Artificial

<220>

<223> Artificial Sequence 1-425

<400> 13

Met Thr Leu Leu Pro Gly Leu Leu Phe Leu Thr Trp Leu His Thr  
1 5 10 15

Cys Leu Ala His His Asp Pro Ser Leu Arg Gly His Pro His Ser  
20 25 30

His Gly Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly  
35 40 45

Gln Ala Pro Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln  
50 55 60

Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His  
65 70 75

Arg Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln Cys Pro Val  
80 85 90

Leu Arg Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln Arg Ser  
95 100 105

Ile Ser Pro Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg Tyr  
110 115 120

Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile

|   |     |  |     |  |     |
|---|-----|--|-----|--|-----|
|   | 125 |  | 130 |  | 135 |
| Asp Ala Arg Thr Gly Arg Glu Thr Ala Ala Leu Asn Ser Val Arg | 140 |  | 145 |  | 150 |
| Leu Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg | 155 |  | 160 |  | 165 |
| Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr | 170 |  | 175 |  | 180 |
| Glu Phe Ile His Val Pro Val Gly Cys Thr Cys Val Leu Pro Arg | 185 |  | 190 |  | 195 |
| Ser Val Pro Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro | 200 |  | 205 |  | 210 |
| Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro | 215 |  | 220 |  | 225 |
| Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val | 230 |  | 235 |  | 240 |
| Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp | 245 |  | 250 |  | 255 |
| Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg | 260 |  | 265 |  | 270 |
| Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr | 275 |  | 280 |  | 285 |
| Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys | 290 |  | 295 |  | 300 |
| Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser | 305 |  | 310 |  | 315 |
| Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro | 320 |  | 325 |  | 330 |
| Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys | 335 |  | 340 |  | 345 |



Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu  
350 355 360

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
365 370 375

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
380 385 390

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
395 400 405

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
410 415 420

Leu Ser Pro Gly Lys  
425

<210> 14

<211> 212

<212> PRT

<213> Homo sapiens

<400> 14

Met Asn Ser Phe Ser Thr Ser Ala Phe Gly Pro Val Ala Phe Ser  
1 5 10 15

Leu Gly Leu Leu Leu Val Leu Pro Ala Ala Phe Pro Ala Pro Val  
20 25 30

Pro Pro Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln  
35 40 45

Pro Leu Thr Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile  
50 55 60

Leu Asp Gly Ile Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser  
65 70 75

Asn Met Cys Glu Ser Ser Lys Glu Ala Leu Ala Glu Asn Asn Leu  
80 85 90

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Leu | Pro | Lys | Met | Ala | Glu | Lys | Asp | Gly | Cys | Phe | Gln | Ser | Gly |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Asn | Glu | Glu | Thr | Cys | Leu | Val | Lys | Ile | Ile | Thr | Gly | Leu | Leu |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Phe | Glu | Val | Tyr | Leu | Glu | Tyr | Leu | Gln | Asn | Arg | Phe | Glu | Ser |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Glu | Glu | Gln | Ala | Arg | Ala | Val | Gln | Met | Ser | Thr | Lys | Val | Leu |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Gln | Phe | Leu | Gln | Lys | Lys | Ala | Lys | Asn | Leu | Asp | Ala | Ile | Thr |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Asp | Pro | Thr | Thr | Asn | Ala | Ser | Leu | Leu | Thr | Lys | Leu | Gln |
|     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Gln | Asn | Gln | Trp | Leu | Gln | Asp | Met | Thr | Thr | His | Leu | Ile | Leu |
|     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ser | Phe | Lys | Glu | Phe | Leu | Gln | Ser | Ser | Leu | Arg | Ala | Leu | Arg |
|     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |

|     |     |
|-----|-----|
| Gln | Met |
|     | 212 |

&lt;210&gt; 15

&lt;211&gt; 320

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gly | Ala | Ala | Arg | Ser | Pro | Pro | Ser | Ala | Val | Pro | Gly | Pro | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Leu | Leu | Leu | Leu | Leu | Leu | Gly | Val | Leu | Ala | Pro | Gly | Gly |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ser | Leu | Arg | Leu | Leu | Asp | His | Arg | Ala | Leu | Val | Cys | Ser | Gln |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gly | Leu | Asn | Cys | Thr | Val | Lys | Asn | Ser | Thr | Cys | Leu | Asp | Asp |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|

|   |     |  |     |  |     |
|---|-----|--|-----|--|-----|
|   | 50  |  | 55  |  | 60  |
| Ser Trp Ile His Pro Arg Asn Leu Thr Pro Ser Ser Pro Lys Asp | 65  |  | 70  |  | 75  |
| Leu Gln Ile Gln Leu His Phe Ala His Thr Gln Gln Gly Asp Leu | 80  |  | 85  |  | 90  |
| Phe Pro Val Ala His Ile Glu Trp Thr Leu Gln Thr Asp Ala Ser | 95  |  | 100 |  | 105 |
| Ile Leu Tyr Leu Glu Gly Ala Glu Leu Ser Val Leu Gln Leu Asn | 110 |  | 115 |  | 120 |
| Thr Asn Glu Arg Leu Cys Val Arg Phe Glu Phe Leu Ser Lys Leu | 125 |  | 130 |  | 135 |
| Arg His His His Arg Arg Trp Arg Phe Thr Phe Ser His Phe Val | 140 |  | 145 |  | 150 |
| Val Asp Pro Asp Gln Glu Tyr Glu Val Thr Val His His Leu Pro | 155 |  | 160 |  | 165 |
| Lys Pro Ile Pro Asp Gly Asp Pro Asn His Gln Ser Lys Asn Phe | 170 |  | 175 |  | 180 |
| Leu Val Pro Asp Cys Glu His Ala Arg Met Lys Val Thr Thr Pro | 185 |  | 190 |  | 195 |
| Cys Met Ser Ser Gly Ser Leu Trp Asp Pro Asn Ile Thr Val Glu | 200 |  | 205 |  | 210 |
| Thr Leu Glu Ala His Gln Leu Arg Val Ser Phe Thr Leu Trp Asn | 215 |  | 220 |  | 225 |
| Glu Ser Thr His Tyr Gln Ile Leu Leu Thr Ser Phe Pro His Met | 230 |  | 235 |  | 240 |
| Glu Asn His Ser Cys Phe Glu His Met His His Ile Pro Ala Pro | 245 |  | 250 |  | 255 |
| Arg Pro Glu Glu Phe His Gln Arg Ser Asn Val Thr Leu Thr Leu | 260 |  | 265 |  | 270 |

Arg Asn Leu Lys Gly Cys Cys Arg His Gln Val Gln Ile Gln Pro  
275 280 285

Phe Phe Ser Ser Cys Leu Asn Asp Cys Leu Arg His Ser Ala Thr  
290 295 300

Val Ser Cys Pro Glu Met Pro Asp Thr Pro Glu Pro Ile Pro Asp  
305 310 315

Tyr Met Pro Leu Trp  
320

<210> 16

<211> 543

<212> DNA

<213> Homo sapiens

<400> 16

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ggggctgggc cagcccagga gcccctaaaag caagaggaag gggcaagggc 100  
ggcctggggc cctggcccct ggccctcacc aggtgccact ggacctgggtg 150  
tcacggatga aaccgtatgc ccgcatggag gagtatgaga ggaacatcga 200  
ggagatgggtg gccagctga ggaacagctc agagctggcc cagagaaagt 250  
gtgaggtcaa cttgcagctg tggatgtcca acaagaggag cctgtctccc 300  
tggggctaca gcatcaacca cgaccccagc cgtatccccg tggacctgcc 350  
ggaggcacgg tgctgtgtc tgggctgtgt gaacccttc accatgcagg 400  
aggaccgcag catggtgagc gtgccggtgt tcagccaggt tcctgtgcgc 450  
cgccgcctct gcccgccacc gcccgcaca gggccttgcc gccagcgcgc 500  
agtcatggag accatcgctg tgggctgcac ctgcatcttc tga 543

<210> 17

<211> 594

<212> DNA

<213> Homo sapiens

<400> 17

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atgacgctcc tccccggcct cctgtttctg acctggctgc acacatgcct 50
ggcccaccat gacctctccc tcagggggca ccccccacagt cacggtacct 100
cacactgcta ctgggtgag gaactgcccc tcggccaggc cccccacac 150
ctgctggctc gaggtgcaa gtgggggcag gctttgcctg tagccctggt 200
gtccagcctg gaggcagcaa gccacagggg gaggcacgag aggccctcag 250
ctacgacca gtgcccgggtg ctgcggccgg aggaggtgtt ggaggcagac 300
accaccagc gtcctatctc accctggaga taccgtgtgg acacggatga 350
ggaccgctat ccacagaagc tggccttcgc cgagtgcctg tgcagaggct 400
gtatcgatgc acggacgggc cgcgagacag ctgcgctcaa ctccgtgcgg 450
ctgctccaga gctgctggt gctgcgccgc cggccctgct cccgcgacgg 500
ctcggggctc cccacacctg gggcctttgc cttccacacc gagttcatcc 550
acgtccccgt cggctgcacc tgcgtgctgc cccgttcagt gtga 594

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<210> 18

<211> 9

<212> PRT

<213> Artificial

<220>

<223> Artificial sequence 1-9

<400> 18

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Gly His His His His His His His
  1             5             9

```

<210> 19

<211> 157

<212> PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Val | Arg | Ser | Ser | Ser | Arg | Thr | Pro | Ser | Asp | Lys | Pro | Val | Ala | His |  |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |  |
| Val | Val | Ala | Asn | Pro | Gln | Ala | Glu | Gly | Gln | Leu | Gln | Trp | Leu | Asn |  |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |  |
| Arg | Arg | Ala | Asn | Ala | Leu | Leu | Ala | Asn | Gly | Val | Glu | Leu | Arg | Asp |  |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |  |
| Asn | Gln | Leu | Val | Val | Pro | Ser | Glu | Gly | Leu | Tyr | Leu | Ile | Tyr | Ser |  |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |  |
| Gln | Val | Leu | Phe | Lys | Gly | Gln | Gly | Cys | Pro | Ser | Thr | His | Val | Leu |  |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |  |
| Leu | Thr | His | Thr | Ile | Ser | Arg | Ile | Ala | Val | Ser | Tyr | Gln | Thr | Lys |  |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |  |
| Val | Asn | Leu | Leu | Ser | Ala | Ile | Lys | Ser | Pro | Cys | Gln | Arg | Glu | Thr |  |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |  |
| Pro | Glu | Gly | Ala | Glu | Ala | Lys | Pro | Trp | Tyr | Glu | Pro | Ile | Tyr | Leu |  |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
| Gly | Gly | Val | Phe | Gln | Leu | Glu | Lys | Gly | Asp | Arg | Leu | Ser | Ala | Glu |  |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
| Ile | Asn | Arg | Pro | Asp | Tyr | Leu | Asp | Phe | Ala | Glu | Ser | Gly | Gln | Val |  |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |
| Tyr | Phe | Gly | Ile | Ile | Ala | Leu |     |     |     |     |     |     |     |     |  |
|     |     |     |     | 155 |     | 157 |     |     |     |     |     |     |     |     |  |

&lt;210&gt; 20

&lt;211&gt; 21

&lt;212&gt; DNA

&lt;213&gt; Artificial

&lt;220&gt;

&lt;223&gt; Artificial sequence 1-21

<400> 20

ctgtacctcg aggggtgcaga g 21

<210> 21

<211> 58

<212> DNA

<213> Artificial

<220>

<223> Artificial sequence 1-58

<400> 21

cccaagcttg ggtcaatgat gatgatgatg atgatgatgc cacaggggca 50

tgtagtcc 58

<210> 22

<211> 328

<212> PRT

<213> Homo sapiens

<400> 22

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Gly | Ala | Ala | Arg | Ser | Pro | Pro | Ser | Ala | Val | Pro | Gly | Pro | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gly | Leu | Leu | Leu | Leu | Leu | Leu | Gly | Val | Leu | Ala | Pro | Gly | Gly |
|     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ser | Leu | Arg | Leu | Leu | Asp | His | Arg | Ala | Leu | Val | Cys | Ser | Gln |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Gly | Leu | Asn | Cys | Thr | Val | Lys | Asn | Ser | Thr | Cys | Leu | Asp | Asp |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Trp | Ile | His | Pro | Arg | Asn | Leu | Thr | Pro | Ser | Ser | Pro | Lys | Asp |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gln | Ile | Gln | Leu | His | Phe | Ala | His | Thr | Gln | Gln | Gly | Asp | Leu |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Pro | Val | Ala | His | Ile | Glu | Trp | Thr | Leu | Gln | Thr | Asp | Ala | Ser |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ile | Leu | Tyr | Leu | Glu | Gly | Ala | Glu | Leu | Ser | Val | Leu | Gln | Leu | Asn |  |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |  |
| Thr | Asn | Glu | Arg | Leu | Cys | Val | Arg | Phe | Glu | Phe | Leu | Ser | Lys | Leu |  |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |  |
| Arg | His | His | His | Arg | Arg | Trp | Arg | Phe | Thr | Phe | Ser | His | Phe | Val |  |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |  |
| Val | Asp | Pro | Asp | Gln | Glu | Tyr | Glu | Val | Thr | Val | His | His | Leu | Pro |  |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |  |
| Lys | Pro | Ile | Pro | Asp | Gly | Asp | Pro | Asn | His | Gln | Ser | Lys | Asn | Phe |  |
|     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |  |
| Leu | Val | Pro | Asp | Cys | Glu | His | Ala | Arg | Met | Lys | Val | Thr | Thr | Pro |  |
|     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |  |
| Cys | Met | Ser | Ser | Gly | Ser | Leu | Trp | Asp | Pro | Asn | Ile | Thr | Val | Glu |  |
|     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |  |
| Thr | Leu | Glu | Ala | His | Gln | Leu | Arg | Val | Ser | Phe | Thr | Leu | Trp | Asn |  |
|     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |  |
| Glu | Ser | Thr | His | Tyr | Gln | Ile | Leu | Leu | Thr | Ser | Phe | Pro | His | Met |  |
|     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |
| Glu | Asn | His | Ser | Cys | Phe | Glu | His | Met | His | His | Ile | Pro | Ala | Pro |  |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |  |
| Arg | Pro | Glu | Glu | Phe | His | Gln | Arg | Ser | Asn | Val | Thr | Leu | Thr | Leu |  |
|     |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |  |
| Arg | Asn | Leu | Lys | Gly | Cys | Cys | Arg | His | Gln | Val | Gln | Ile | Gln | Pro |  |
|     |     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |  |
| Phe | Phe | Ser | Ser | Cys | Leu | Asn | Asp | Cys | Leu | Arg | His | Ser | Ala | Thr |  |
|     |     |     |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |  |
| Val | Ser | Cys | Pro | Glu | Met | Pro | Asp | Thr | Pro | Glu | Pro | Ile | Pro | Asp |  |
|     |     |     |     | 305 |     |     |     |     | 310 |     |     |     |     | 315 |  |



Tyr Met Pro Leu Trp His His His His His His His His  
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<210> 23

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<213> Artificial

<220>

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Lys Gly Gln Gly Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln  
                           20                          25                          30

Val Pro Leu Asp Leu Val Ser Arg Met Lys Pro Tyr Ala Arg Met  
                           35                          40                          45

Glu Glu Tyr Glu Arg Asn Ile Glu Glu Met Val Ala Gln Leu Arg  
                           50                          55                          60

Asn Ser Ser Glu Leu Ala Gln Arg Lys Cys Glu Val Asn Leu Gln  
                           65                          70                          75

Leu Trp Met Ser Asn Lys Arg Ser Leu Ser Pro Trp Gly Tyr Ser  
                           80                          85                          90

Ile Asn His Asp Pro Ser Arg Ile Pro Val Asp Leu Pro Glu Ala  
                           95                          100                          105

Arg Cys Leu Cys Leu Gly Cys Val Asn Pro Phe Thr Met Gln Glu  
                           110                          115                          120

Asp Arg Ser Met Val Ser Val Pro Val Phe Ser Gln Val Pro Val  
                           125                          130                          135

Arg Arg Arg Leu Cys Pro Pro Pro Pro Arg Thr Gly Pro Cys Arg  
                           140                          145                          150

Gln Arg Ala Val Met Glu Thr Ile Ala Val Gly Cys Thr Cys Ile

|   |     |     |     |
|---|-----|-----|-----|
|   | 155 | 160 | 165 |
| Phe Gly His His His His His His His His |     |     |     |
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| 1   | 5   | 10  | 15  |
| Cys Leu Ala His His Asp Pro Ser Leu Arg Gly His Pro His Ser |     |     |     |
|   | 20  | 25  | 30  |
| His Gly Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly |     |     |     |
|   | 35  | 40  | 45  |
| Gln Ala Pro Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln |     |     |     |
|   | 50  | 55  | 60  |
| Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His |     |     |     |
|   | 65  | 70  | 75  |
| Arg Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln Cys Pro Val |     |     |     |
|   | 80  | 85  | 90  |
| Leu Arg Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln Arg Ser |     |     |     |
|   | 95  | 100 | 105 |
| Ile Ser Pro Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg Tyr |     |     |     |
|   | 110 | 115 | 120 |
| Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile |     |     |     |
|   | 125 | 130 | 135 |
| Asp Ala Arg Thr Gly Arg Glu Thr Ala Ala Leu Asn Ser Val Arg |     |     |     |
|   | 140 | 145 | 150 |

Leu Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg  
155 160 165

Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr  
170 175 180

Glu Phe Ile His Val Pro Val Gly Cys Thr Cys Val Leu Pro Arg  
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Ser Val Gly His His His His His His His His  
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<210> 25

<211> 271

<212> PRT

<213> Homo sapiens

<400> 25

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Asn Gln Lys Ser Phe Tyr His Val Ser Tyr Gly Pro Leu His Glu  
35 40 45

Gly Cys Met Asp Gln Ser Val Ser Leu Ser Ile Ser Glu Thr Ser  
50 55 60

Lys Thr Ser Lys Leu Thr Phe Lys Glu Ser Met Val Val Val Ala  
65 70 75

Thr Asn Gly Lys Val Leu Lys Lys Arg Arg Leu Ser Leu Ser Gln  
80 85 90

Ser Ile Thr Asp Asp Asp Leu Glu Ala Ile Ala Asn Asp Ser Glu  
95 100 105

Glu Glu Ile Ile Lys Pro Arg Ser Ala Pro Phe Ser Phe Leu Ser  
110 115 120

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Val | Lys | Tyr | Asn | Phe | Met | Arg | Ile | Ile | Lys | Tyr | Glu | Phe | Ile |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Asn | Asp | Ala | Leu | Asn | Gln | Ser | Ile | Ile | Arg | Ala | Asn | Asp | Gln |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Leu | Thr | Ala | Ala | Ala | Leu | His | Asn | Leu | Asp | Glu | Ala | Val | Lys |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Asp | Met | Gly | Ala | Tyr | Lys | Ser | Ser | Lys | Asp | Asp | Ala | Lys | Ile |
|     |     |     |     | 170 |     |     |     |     | 175 |     |     |     |     | 180 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Val | Ile | Leu | Arg | Ile | Ser | Lys | Thr | Gln | Leu | Tyr | Val | Thr | Ala |
|     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |     | 195 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Asp | Glu | Asp | Gln | Pro | Val | Leu | Leu | Lys | Glu | Met | Pro | Glu | Ile |
|     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     | 210 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Lys | Thr | Ile | Thr | Gly | Ser | Glu | Thr | Asn | Leu | Leu | Phe | Phe | Trp |
|     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     | 225 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Thr | His | Gly | Thr | Lys | Asn | Tyr | Phe | Thr | Ser | Val | Ala | His | Pro |
|     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Leu | Phe | Ile | Ala | Thr | Lys | Gln | Asp | Tyr | Trp | Val | Cys | Leu | Ala |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Pro | Pro | Ser | Ile | Thr | Asp | Phe | Gln | Ile | Leu | Glu | Asn | Gln |
|     |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |

Ala  
271

<210> 26

<211> 177

<212> PRT

<213> Homo sapiens

<400> 26

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| Met | Glu | Ile | Cys | Arg | Gly | Leu | Arg | Ser | His | Leu | Ile | Thr | Leu | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

Leu Phe Leu Phe His Ser Glu Thr Ile Cys Arg Pro Ser Gly Arg

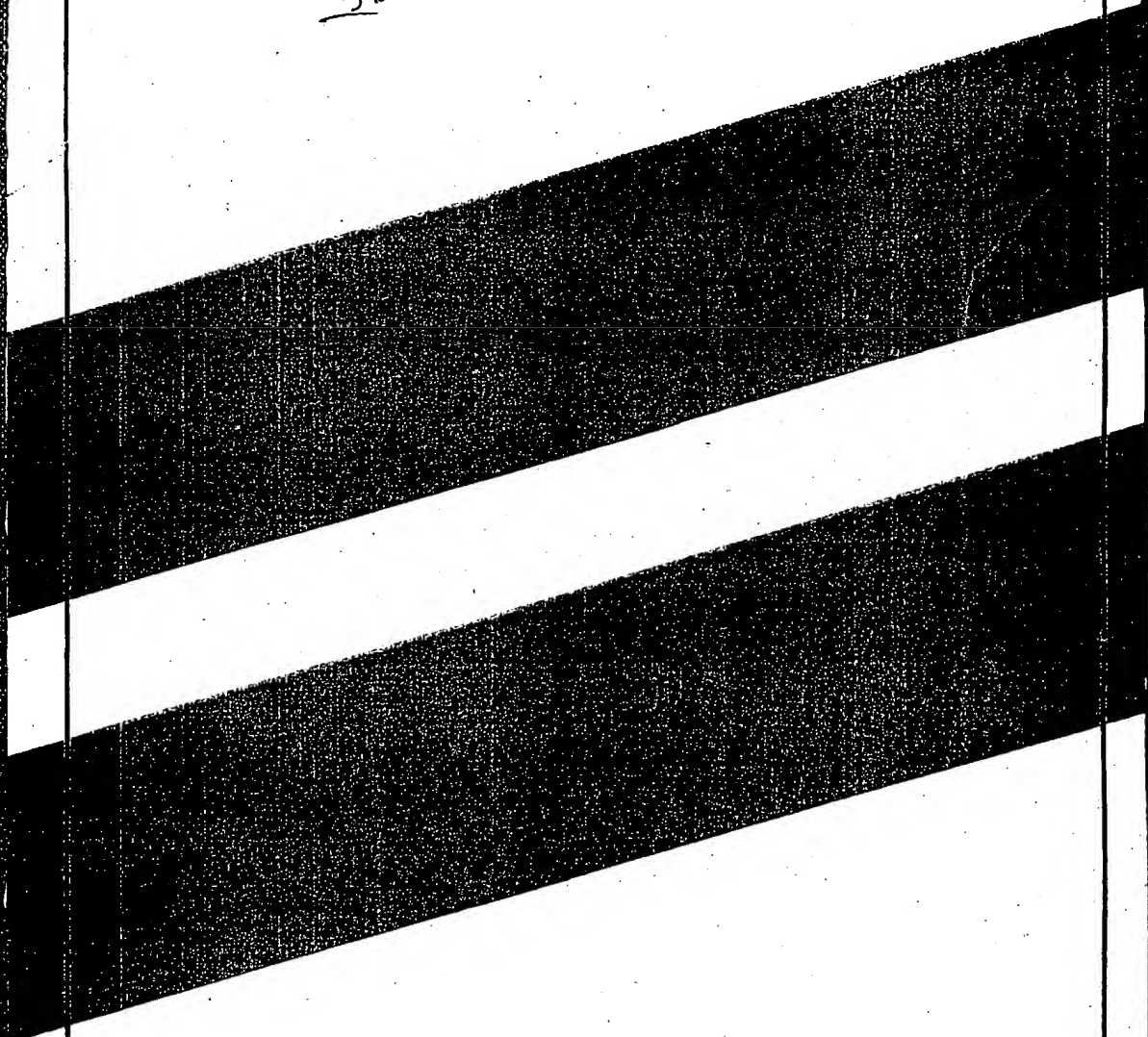
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|     |     |     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30 |
| Lys | Ser | Ser | Lys | Met | Gln | Ala | Phe | Arg | Ile | Trp | Asp | Val | Asn | Gln |    |
|     |     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |    |
| Lys | Thr | Phe | Tyr | Leu | Arg | Asn | Asn | Gln | Leu | Val | Ala | Gly | Tyr | Leu |    |
|     |     |     |     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |    |
| Gln | Gly | Pro | Asn | Val | Asn | Leu | Glu | Glu | Lys | Ile | Asp | Val | Val | Pro |    |
|     |     |     |     | 65  |     |     |     |     | 70  |     |     |     |     | 75  |    |
| Ile | Glu | Pro | His | Ala | Leu | Phe | Leu | Gly | Ile | His | Gly | Gly | Lys | Met |    |
|     |     |     |     | 80  |     |     |     |     | 85  |     |     |     |     | 90  |    |
| Cys | Leu | Ser | Cys | Val | Lys | Ser | Gly | Asp | Glu | Thr | Arg | Leu | Gln | Leu |    |
|     |     |     |     | 95  |     |     |     |     | 100 |     |     |     |     | 105 |    |
| Glu | Ala | Val | Asn | Ile | Thr | Asp | Leu | Ser | Glu | Asn | Arg | Lys | Gln | Asp |    |
|     |     |     |     | 110 |     |     |     |     | 115 |     |     |     |     | 120 |    |
| Lys | Arg | Phe | Ala | Phe | Ile | Arg | Ser | Asp | Ser | Gly | Pro | Thr | Thr | Ser |    |
|     |     |     |     | 125 |     |     |     |     | 130 |     |     |     |     | 135 |    |
| Phe | Glu | Ser | Ala | Ala | Cys | Pro | Gly | Trp | Phe | Leu | Cys | Thr | Ala | Met |    |
|     |     |     |     | 140 |     |     |     |     | 145 |     |     |     |     | 150 |    |
| Glu | Ala | Asp | Gln | Pro | Val | Ser | Leu | Thr | Asn | Met | Pro | Asp | Glu | Gly |    |
|     |     |     |     | 155 |     |     |     |     | 160 |     |     |     |     | 165 |    |
| Val | Met | Val | Thr | Leu | Phe | Tyr | Phe | Gln | Glu | Asp | Glu |     |     |     |    |
|     |     |     |     | 170 |     |     |     |     | 175 |     | 177 |     |     |     |    |



|                      |          |
|----------------------|----------|
|                      |          |
| Class                | Subclass |
| ISSUE CLASSIFICATION |          |
| SCANNED 0            |          |

PROVISIONAL  
APPLICATION  
NUMBER

*Ebner*  
1340



SCANNED *HT*  
*QC SM* *[Signature]*

PATENT APPLICATION



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Received  
or  
Mailed

1. Application \_\_\_\_\_ papers.

2. *Request for*

3. \_\_\_\_\_

4. \_\_\_\_\_

11. \_\_\_\_\_

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32. \_\_\_\_\_

| POSITION    | ID NO. | DATE   |
|-------------|--------|--------|
| CLASSIFIER  |        |        |
| EXAMINER    | 66-548 | 7/2/78 |
| TYPIST      |        |        |
| VERIFIER    | ✓      | ✓      |
| CORPS CORR. |        |        |
| SPEC. HAND  |        |        |
| FILE MAINT  |        |        |
| DRAFTING    |        |        |



PATENT APPLICATION SERIAL NO. \_\_\_\_\_

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE  
FEE RECORD SHEET

04/05/1998 JMTTS 00000071-003425 60007340  
01 FC:114 150.00 CH

71479 U.S. PTO



05/29/98

866250-0462809

U.S. PATENT AND TRADEMARK OFFICE  
PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

|            |                |   |   |
|------------|----------------|---|---|
| Docket No. | <b>PF470PP</b> | Type a plus sign (+)<br>inside this box → | + |
|------------|----------------|---|---|

## ♦ INVENTOR (S) / APPLICANT (S)

| Last Name | First Name | Middle Initial | Residence (City and Either State or Foreign Country) |
|-----------|------------|----------------|--|
| Ebner     | Reinhard   |                | Gaithersburg, MD                                     |
| Ruben     | Steven     |                | Olney, MD  |
|           |            |                |  |

## ♦ TITLE OF THE INVENTION (280 characters max)

Interleukins-21 and 22

## ♦ CORRESPONDENCE ADDRESS

A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582) and Kenley K. Hoover (Reg. No. 40,302) of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20850. Ph: 301-309-8504 Fax: 301-309-8439

|       |          |          |       |         |      |
|-------|----------|----------|-------|---------|------|
| State | Maryland | Zip Code | 20850 | Country | U.S. |
|-------|----------|----------|-------|---------|------|

## ♦ ENCLOSED APPLICATION PARTS (check all that apply)

- |   |              |      |  |                |         |
|---|--------------|------|--|----------------|---------|
| <input checked="" type="checkbox"/> Specification | No. of Pages | 85   | <input checked="" type="checkbox"/> 36 Claims        | No. of pages:  | 6       |
|   | Pages:       | 1-85 |  | Pages:         | 100-105 |
| <input checked="" type="checkbox"/> Abstract      | No. of Pages | 1    | <input checked="" type="checkbox"/> Sequence Listing | No. of pages:  | 14      |
|   | Page:        | 106  |  | Pages:         | 86-99   |
|   |              |      | <input checked="" type="checkbox"/> 5 Figures        | No. of sheets: | 6       |

## ♦ METHOD OF PAYMENT (check one)

- |   |                           |          |
|---|---------------------------|----------|
| <input type="checkbox"/> A check or money order is enclosed to cover the Provisional filing fee   | PROVISIONAL               |          |
| <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number 08-3425 | FILING FEE<br>AMOUNT (\$) | \$150.00 |

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE:

Date 5/27/98

TYPE OR PRINTED NAME

A. Anders Brookes

REGISTRATION NO.  
(If appropriate)

(Reg. No. 36,373)

☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

AAB/JK/rdh

## Interleukins-21 and 22

### *Field of the Invention*

The present invention relates to two novel human genes, each of which encodes a polypeptide which is a member of the Interleukin family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide  
5 named Interleukin-21, or "IL-21". The present invention also relates to a polynucleotide encoding a novel human polypeptide named Interleukin-22, or "IL-22". This invention also relates to IL-21 and IL-22 polypeptides, as well as vectors, host cells, antibodies directed to IL-21 and IL-22 polypeptides, and the recombinant  
10 methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the Immune system, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of IL-21 and IL-22 activity.

### *Background of the Invention*

Cytokines typically exert their respective biochemical and physiological effects by binding to specific receptor molecules. Receptor binding then stimulates specific signal transduction pathways (Kishimoto, T., *et al.*, *Cell* 76:253-262 (1994)). The specific interactions of cytokines with their receptors are often the primary regulators of a wide variety of cellular processes including activation, proliferation, and  
20 differentiation (Arai, K. -I, *et al.*, *Ann. Rev. Biochem.* 59:783-836 (1990); Paul, W. E. and Seder, R. A., *Cell* 76:241-251 (1994)).

Human interleukin (IL)-17, a closely related homolog of the molecules of the present invention, was only recently identified. IL-17 is a 155 amino acid polypeptide which was molecularly cloned from a CD4+ T-cell cDNA library (Yao, Z., *et al.*, *J.*  
25 *Immunol.* 155:5483-5486 (1995)). The IL-17 polypeptide contains an N-terminal signal peptide and contains approximately 72% identity at the amino acid level with a T-cell trophic herpesvirus saimiri (HVS) gene designated HVS13. High levels of IL-17 are secreted from CD4-positive primary peripheral blood leukocytes (PBL) upon stimulation (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Treatment of fibroblasts  
30 with IL-17, HVS13, or another murine homologue, designated CTLA8, activate signal transduction pathways and result in the stimulation of the NF- $\kappa$ B transcription factor family, the secretion of IL-6, and the costimulation of T-cell proliferation (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)).

An HVS13-Fc fusion protein was used to isolate a murine IL-17 receptor  
35 molecule which does not appear to belong to any of the previously described cytokine

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receptor families (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). The murine IL-17 receptor (mIL-17R) is predicted to encode a type I transmembrane protein of 864 amino acids with an apparent molecular mass of 97.8 kDa. mIL-17R is predicted to possess an N-terminal signal peptide with a cleavage site between alanine-31 and serine-32.

- 5 The molecule also contains a 291 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 521 amino acid cytoplasmic tail. A soluble recombinant IL-17R molecule consisting of 323 amino acids of the extracellular domain of IL-17R fused to the Fc portion of human immunoglobulin IgG1 was able to significantly inhibit IL-17-induced IL-6 production by murine NIH-3T3 cells (*supra*).

- 10 Interestingly, the expression of the IL-17 gene is highly restricted. It is typically observed primarily in activated T-lymphocyte memory cells (Broxmeyer, H. J. *Exp. Med.* 183:2411-2415 (1996); Fossiez, F., *et al.*, *J. Exp. Med.* 183:2593-2603 (1996)). Conversely, the IL-17 receptor appears to be expressed in a large number of cells and tissues (Rouvier, E., *et al.*, *J. Immunol.* 150:5445-5456 (1993); Yao, Z., *et al.*, *J. Immunol.* 155:5483-5486 (1995)). It remains to be seen, however, if IL-17  
15 itself can play an autocrine role in the expression of IL-17. IL-17 has been implicated as a causative agent in the expression of IL-6, IL-8, G-CSF, Prostaglandin E (PGE<sub>2</sub>), and intracellular adhesion molecule (ICAM)-1 (Fossiez, F., *supra*; Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Each of these molecules possesses highly relevant and  
20 potentially therapeutically valuable properties. For instance, IL-6 is involved in the regulation of hematopoietic stem and progenitor cell growth and expansion (Ikebuchi, K., *et al.*, *Proc. Natl. Acad. Sci. USA* 84:9035-9039 (1987); Gentile, P. and Broxmeyer, H. E. *Ann. N.Y. Acad. Sci. USA* 628:74-83 (1991)). IL-8 exhibits a myelosuppressive activity for stem cells and immature subsets of myeloid progenitors  
25 (Broxmeyer, H. E., *et al.*, *Ann. Hematol.* 71:235-246 (1995); Daly, T. J., *et al.*, *J. Biol. Chem.* 270:23282-23292 (1995)). G-CSF acts both early and late to activate and stimulate hematopoiesis in general, and more specifically on neutrophil hematopoiesis, while PGE<sub>2</sub> enhances erythropoiesis, suppresses lymphopoiesis and myelopoiesis in general, and strongly suppresses monocytopoiesis (Broxmeyer, H. E. *Amer. J. Ped. Hematol./Oncol.* 14:22-30 (1992); Broxmeyer, H. E. and Williams, D. E. *CRC Crit. Rev. Oncol./Hematol.* 8:173-226 (1988)).  
30

- 35 Thus, there is a need for polypeptides that function as immunoregulatory molecules and, thereby, modulate the transfer of an extracellular signal ultimately to the nucleus of the cell, since disturbances of such regulation may be involved in disorders relating to cellular activation, hemostasis, angiogenesis, tumor metastasis, cellular migration and ovulation, as well as neurogenesis. Therefore, there is a need for

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identification and characterization of such human polypeptides which can play a role in detecting, preventing, ameliorating or correcting such disorders.

### ***Summary of the Invention***

The present invention relates to novel polynucleotides and the encoded  
 5 polypeptides of IL-21 and IL-22. Moreover, the present invention relates to vectors,  
 host cells, antibodies, and recombinant methods for producing the polypeptides and  
 polynucleotides. Also provided are diagnostic methods for detecting disorders related  
 to the polypeptides, and therapeutic methods for treating such disorders. The invention  
 further relates to screening methods for identifying binding partners of IL-21 and  
 10 IL-22.

### ***Brief Description of the Drawings***

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) and the deduced amino  
 acid sequence (SEQ ID NO:2) of IL-21. The locations of conserved Domains I-IV (see  
 below) are underlined and labeled as such.

15 Figure 2 shows the nucleotide sequence (SEQ ID NO:3) and the deduced amino  
 acid sequence (SEQ ID NO:4) of IL-22. The locations of conserved Domains I-IV (see  
 below) are underlined and labeled as such. The locations of two potential N-linked  
 glycosylation sites are identified by a bolded asparagine symbol (N) accompanied by a  
 bolded pound sign (#) located above the initial nucleotide of the codon encoding the  
 20 corresponding asparagine.

Figure 3 shows the regions of identity between the amino acid sequence of the  
 IL-21 and IL-22 proteins, the amino acid sequence of IL-20 (disclosed in copending  
 U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ  
 ID NO:8), and the translation products of human Interleukin-17 (ATCC Accession No.  
 25 U32659; SEQ ID NO:5), mouse Interleukin-17 (ATCC Accession No. U43088; SEQ  
 ID NO:6), and viral Interleukin-17 (ATCC Accession No. X64346; SEQ ID NO:7), as  
 determined by the MegAlign component of the computer program DNA\*Star  
 (DNASTAR, Inc.) using the default parameters.

Figure 4 shows an analysis of the IL-21 amino acid sequence. Alpha, beta, turn  
 30 and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible  
 regions; antigenic index and surface probability are shown. In the "Antigenic Index" or  
 "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic  
 regions of the IL-21 protein, that is, regions from which epitope-bearing peptides of the  
 invention can be determined. The domains defined by these graphs are contemplated by  
 35 the present invention.

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Figure 5 shows an analysis of the IL-22 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the IL-22 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. The domains defined by these graphs are contemplated by the present invention.

### *Detailed Description*

#### Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

In the present invention, a "secreted" IL-21 or IL-22 protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as an IL-21 or IL-22 protein released into the extracellular space without necessarily containing a signal sequence. If the IL-21 or IL-22 secreted protein is released into the extracellular space, the IL-21 or IL-22 secreted protein can undergo extracellular processing to produce a "mature" IL-21 or IL-22 protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, an IL-21 or IL-22 "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or in SEQ ID NO:3, respectively, or the cDNA contained within the respective clones deposited with the ATCC. For example, the IL-21 or IL-22 polynucleotide can contain the nucleotide sequence of the full-length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, an IL-21 or IL-22 "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full-length IL-21 sequence identified as SEQ ID NO:1 was generated by overlapping sequences contained in multiple clones (contig

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analysis). A representative clone containing all or most of the sequence for SEQ ID NO:1 (designated HTGED19) was deposited with the American Type Culture Collection ("ATCC") on March 5, 1998, and was given the ATCC Deposit Number 209666. In addition, and also in the present invention, the full-length IL-22 sequence identified as SEQ ID NO:3 was also generated by overlapping sequences contained in multiple clones (contig analysis). Likewise, a representative clone containing all or most of the sequence for SEQ ID NO:3 (designated HFPBX96) was also deposited with the ATCC on March 5, 1998, and was given the ATCC Deposit Number 209665. The ATCC is located at 10801 University Blvd., Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

An IL-21 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the cDNA within the deposited clone. Further, An IL-22 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:3, the complement thereof, or the cDNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the IL-21 and the IL-22 polynucleotides at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include

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Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

- 5       Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a polyA+ stretch or the complement thereof (e.g., practically any
- 10       double-stranded cDNA clone).

- The IL-21 and IL-22 polynucleotides can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the IL-21 and IL-22 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single-
- 15       and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the IL-21 polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA.
- 20       IL-21 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

- 25       IL-21 and IL-22 polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The IL-21 and IL-22 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art.
- 30       Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the IL-21 and IL-22 polypeptides, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given
- 35       IL-21 or IL-22 polypeptide. Also, a given IL-21 or IL-22 polypeptide may contain many types of modifications. IL-21 or IL-22 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without

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branching. Cyclic, branched, and branched cyclic IL-21 and IL-22 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter, *et al.*, *Meth. Enzymol.* **182**:626-646 (1990); Rattan, *et al.*, *Ann. NY Acad. Sci.* **663**:48-62 (1992)).

"SEQ ID NO:1" refers to an IL-21 polynucleotide sequence while "SEQ ID NO:2" refers to an IL-21 polypeptide sequence. Likewise, "SEQ ID NO:3" refers to an IL-22 polynucleotide sequence while "SEQ ID NO:4" refers to an IL-22 polypeptide sequence.

An IL-21 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-21 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In addition, an IL-22 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-22 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In the case where dose-dependency does exist, it need not be identical to that of the IL-21 or IL-22 polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the IL-21 or IL-22 polypeptides (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the IL-21 polypeptide).

#### IL-21 and IL-22 Polynucleotides and Polypeptides

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Clone HTGED19, encoding IL-21, was isolated from a cDNA library derived from apoptotic T-cells. This clone contains the entire coding region identified as SEQ ID NO:2. The deposited clone contains a cDNA having a total of 705 nucleotides, which encodes a partial predicted open reading frame of 87 amino acid residues (see Figure 1). The partial open reading frame begins at a point in the complete IL-21 ORF such that the "G" in position 1 of SEQ ID NO:1 is actually in position 3 of a coding triplet. As such, the partial predicted IL-21 polypeptide sequence is shown beginning in-frame with an alanine residue at position 1 of SEQ ID NO:2. The alanine residue at position 1 of SEQ ID NO:2 is encoded by nucleotides 2-4 of the nucleotide sequence shown as SEQ ID NO:1. The ORF shown as SEQ ID NO:2 ends at a stop codon at nucleotide position 263-265 of the nucleotide sequence shown as SEQ ID NO:1. The predicted molecular weight of the partial IL-21 protein should be about 9,558 Daltons.

An initial BLAST analysis of the expression of the IL-21 cDNA sequence against the HGS EST database has also revealed a highly specific expression of this cDNA clone. In such an analysis, the HTGED19 cDNA sequence appears to be found only in apoptotic T-cells. Thus, IL-21 appears to be expressed in a highly restricted pattern limited to apoptotic T-cells, and, for example, other subpopulations of lymphocytes or other cells in a state of activation or quiescence.

Clone HFPBX96, encoding IL-22, was isolated from a cDNA library derived from epileptic frontal cortex. This clone contains the entire coding region identified as SEQ ID NO:4. The deposited clone contains a cDNA having a total of 1,642 nucleotides, which encodes a partial predicted open reading frame of 160 amino acid residues (see Figure 2). The partial open reading frame begins at a point in the complete IL-22 ORF such that the "G" in position 1 of SEQ ID NO:3 is actually in position two of a coding triplet. As such, the partial predicted IL-22 polypeptide sequence is shown beginning in-frame with an asparagine residue at position 1 of SEQ ID NO:4. The asparagine residue at position 1 of SEQ ID NO:4 is encoded by nucleotides 3-5 of the nucleotide sequence shown as SEQ ID NO:3. The ORF shown as SEQ ID NO:4 ends at a stop codon at nucleotide position 483-485 of the nucleotide sequence shown as SEQ ID NO:3. The predicted molecular weight of the partial IL-22 protein should be about 17,436 Daltons.

Using BLAST and MegAlign analyses, SEQ ID NO:2 and SEQ ID NO:4 were each found to be highly homologous to several members of the Interleukin family. Particularly, SEQ ID NO:2 and SEQ ID NO:4 contain at least four domains homologous to the translation products of the human mRNA for Interleukin (IL)-20 (copending U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ ID NO:8), IL-17 (ATCC Accession No. U32659; SEQ ID NO:5; see also

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Figure 3), the murine mRNA for Interleukin (IL)-17 (ATCC Accession No. U43088; SEQ ID NO:6; see also Figure 3), and the human viral mRNA for Interleukin (IL)-17 (ATCC Accession No. X64346; SEQ ID NO:7; see also Figure 3). Specifically, the molecules of the present invention, in particular, SEQ ID NO:2 and SEQ ID NO:4, share a high degree of sequence identity with IL-20, IL-17, mIL-17, and vIL-17 in the following conserved domains: (a) a predicted NXDPXRY domain (where X represents any amino acid) located at about amino acids valine-3 to proline-11 of SEQ ID NO:2, serine-57 to proline-64 of SEQ ID NO:4, and asparagine-79 to proline-86 of the human IL-17 amino acid sequence (SEQ ID NO:5); (b) a predicted CLCXGC domain (where X represents any amino acid) located at about amino acids cysteine-19 to cysteine-24 of SEQ ID NO:2, cysteine-72 to cysteine-77 of SEQ ID NO:4, and cysteine-94 to cysteine-99 of the human IL-17 amino acid sequence (SEQ ID NO:5); (c) a predicted LVLRRXP domain (where X represents any amino acid) located at about amino acids leucine-46 to proline-52 of SEQ ID NO:2, valine-99 to proline-105 of SEQ ID NO:4, and leucine-120 to proline-126 of the human IL-17 amino acid sequence (SEQ ID NO:5); and (d) a predicted VXVGCTCV domain (where X represents any amino acid) located at about amino acids valine-75 to valine-82 of SEQ ID NO:2, isoleucine-121 to valine-128 of SEQ ID NO:4, and valine-140 to valine-147 of the human IL-17 amino acid sequence (SEQ ID NO:5). These polypeptide fragments of IL-21 are specifically contemplated in the present invention. Because each of these IL-17 and IL-17-like molecules is thought to be important immunoregulatory molecules, the homology between these IL-17 and IL-17-like molecules and IL-21 and IL-22 suggests that IL-21 and IL-22 may also be important immunoregulatory molecules.

Moreover, based on their apparent sequence identities with IL-17 and IL-20 (see Figure 3), the full-length IL-21 and IL-22 polypeptides are each likely to have an amino terminal secretory signal peptide leader sequence. Since the present invention appears to be partial cDNA clones of the IL-21 and IL-22 molecules, it is also contemplated that the translation products of the present invention will be caused to enter the cellular secretory pathway by virtue of being expressed as a fusion proteins comprising several different portions of the N-terminus of the IL-20 molecule of copending U.S. Provisional Application Serial No. 60/060,140 fused to the known coding sequence of the IL-21 or IL-22 molecules of the present invention. Such expression constructs will secrete hybrid IL-20/IL-21 or IL-20/IL-22 molecules from the host cell. The mature IL-21 protein used in these fusion proteins encompasses about amino acids 1-145, while the IL-20/21 fusion protein encompasses about the 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-145 of IL-21. These

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polypeptide fragments of IL-21 are specifically contemplated in the present invention. In addition, the mature IL-22 protein used to generate these fusion proteins encompasses about amino acids 1-160, while the IL-20/22 fusion protein encompasses about the 95, 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-160 of IL-22. These polypeptide fragments of IL-22 are specifically contemplated in the present invention.

The IL-21 and IL-22 nucleotide sequences identified as SEQ ID NO:1 and SEQ ID NO:3, respectively, were assembled from partially homologous ("overlapping") sequences obtained from the deposited clones. The overlapping sequences were assembled into two single contiguous sequences of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in two final sequences identified as SEQ ID NO:1 and SEQ ID NO:3.

Therefore, SEQ ID NO:1 and the translated SEQ ID NO:2, and SEQ ID NO:3 and the translated SEQ ID NO:4, are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1 and SEQ ID NO:3 are useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1, SEQ ID NO:3, or the cDNA contained in the respective deposited cDNA clones. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 and SEQ ID NO:4 may be used to generate antibodies which bind specifically to IL-21 and IL-22, respectively.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, but also a sample of plasmid DNA containing a human cDNA of IL-21 deposited with the ATCC. In addition, the present invention also provides not only the generated nucleotide sequence identified as SEQ ID NO:3 and the predicted translated amino acid sequence identified as SEQ ID NO:4, but

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also a sample of plasmid DNA containing a human cDNA of IL-22 deposited with the ATCC. Accordingly, the nucleotide sequence of the deposited IL-21 and IL-22 clones can be readily determined by sequencing the deposited clone in accordance with known methods. The predicted IL-21 and IL-22 amino acid sequences can then be verified  
5 from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human IL-21 or IL-22 cDNAs, collecting the protein, and determining its sequence.

The present invention also relates to the IL-21 gene corresponding to SEQ ID  
10 NO:1, SEQ ID NO:2, or the deposited clone which encodes IL-21. The present invention further relates to the IL-22 gene corresponding to SEQ ID NO:3, SEQ ID NO:4, or the deposited clone which encodes IL-22. The IL-21 and IL-22 genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed  
15 sequences and identifying or amplifying the IL-21 and IL-22 genes from appropriate sources of genomic material.

Also provided in the present invention are species homologs of IL-21 and IL-22. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source  
20 for the desired homolog.

The IL-21 and IL-22 polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well  
25 understood in the art.

The IL-21 and IL-22 polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein. It is often advantageous to include an additional amino acids which comprise secretory or leader sequences, pro-sequences, sequences which aid in purification, such as  
30 multiple histidine residues, or an additional sequence for stability during recombinant production.

IL-21 and IL-22 polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of an IL-21 or IL-22 polypeptide, including the secreted polypeptide, can be substantially purified by  
35 the one-step method described in the publication by Smith and Johnson (*Gene* 67:31-40 (1988)). IL-21 and IL-22 polypeptides also can be purified from natural or

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recombinant sources using antibodies of the invention raised against the IL-21 and IL-22 proteins, respectively, in methods which are well known in the art.

### Polynucleotide and Polypeptide Variants

5 "Variant" refers to a polynucleotide or polypeptide differing from the IL-21 and IL-22 polynucleotides or polypeptides, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the IL-21 and IL-22 polynucleotide or polypeptide.

10 By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the IL-21 or IL-22 polypeptides. In other words, to obtain a polynucleotide having a nucleotide sequence 15 at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be inserted, deleted or substituted with another nucleotide. The query sequence may be an entire sequence shown of SEQ ID NO:1 or SEQ ID NO:3, the ORF (open reading frame) of either IL-21 or IL-22, or any fragment specified as described herein.

20 As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from) a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence 25 alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting (uridine residues (U) to thymidine residues (T). The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, 30 whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, but not because of internal deletions, a manual correction must be made to the

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results. This is because the FASTDB algorithm does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence ((number of bases at the 5' and 3' ends not matched)/(total number of bases in the query sequence)), so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence which is, at least, for example, 95% "identical" to (or 5% different from) a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (insertions and deletions are collectively referred to as "indels" in the art) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal

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positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

- As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from), for instance, the amino acid sequences shown in SEQ ID NO:2, or that shown in SEQ ID NO:4, or to the amino acid sequence encoded by deposited cDNA clones, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity.
- Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.
- If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

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For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence), so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The IL-21 and IL-22 variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. IL-21 and IL-22 polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring IL-21 and IL-22 variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the IL-21 and IL-22 polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron and coworkers reported variant KGF proteins having heparin

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binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues (*J. Biol. Chem.* 268:2984-2988 (1993)). Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobéli, *et al.*, *J. Biotechnol.* 7:199-216 (1988)).

5 In the present case, since the IL-21 and IL-22 proteins of the invention are highly related to the Interleukin-17-like polypeptide family, deletions of N-terminal amino acids up to the cysteine at position 19 of SEQ ID NO:2 and up to the cysteine at position 29 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further N-terminal deletions including the cysteine-19 residue in SEQ ID NO:2 and the  
10 cysteine-29 residue in SEQ ID NO:4 would not be expected to retain such biological activities because it is likely that these residues are required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

15 However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the  
20 N-termini of the respective proteins. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

25 Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the cysteine residue at position number 19, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4,  
30 up to the cysteine residue at position number 29, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n-87 of SEQ ID NO:2, where n is an integer in the range of 1 to 18, and 19 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for the  
35 receptor binding activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues n-160 of SEQ ID NO:4, where n is an integer in the range of 1 to 28, and 29 is the position of the first residue

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from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for the receptor binding activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-87, 2-87, 3-87, 4-87, 5-87, 6-87, 7-87, 8-87, 9-87, 10-87, 11-87, 12-87, 13-87, 14-87, 15-87, 16-87, 17-87, 18-87, and 19-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided. The invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-160, 2-160, 3-160, 4-160, 5-160, 6-160, 7-160, 8-160, 9-160, 10-160, 11-160, 12-160, 13-160, 14-160, 15-160, 16-160, 17-160, 18-160, 19-160, 20-160, 21-160, 22-160, 23-160, 24-160, 25-160, 26-160, 27-160, 28-160, and 29-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

In addition, since the IL-21 and IL-22 proteins of the invention are highly related to the IL-17-like polypeptide family, deletions of C-terminal amino acids up to the leucine at position 83 of SEQ ID NO:2 and up to the proline at position 129 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further C-terminal deletions including the leucine residue at position 83 of SEQ ID NO:2 and the proline at position 129 of SEQ ID NO:4 would not be expected to retain such biological activities since these residues are in the beginning of the conserved domain required for biological activities.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the leucine residue at position 83 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the proline residues at position 129 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence

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of residues 1-m of the amino acid sequence in SEQ ID NO:2, where m is any integer in the range of 83 to 87, and residue 82 is the position of the first residue from the C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for activity of the IL-21 protein. In addition, the present invention also

5 provides polypeptides having the amino acid sequence of residues 1-m of the amino acid sequence in SEQ ID NO:4, where m is any integer in the range of 129 to 160, and residue 128 is the position of the first residue from the C-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for activity of the IL-22 protein.

10 More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-83, 1-84, 1-85, 1-86, and 1-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided. The present invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-129, 1-130, 1-131, 1-132, 1-133, 1-134, 1-135,  
15 1-136, 1-137, 1-138, 1-139, 1-140, 1-141, 1-142, 1-143, 1-144, 1-145, 1-146, 1-147, 1-148, 1-149, 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 1-156, 1-157, 1-158, 1-159, and 1-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids  
20 deleted from both the amino and the carboxyl termini of IL-21, which may be described generally as having residues n-m of SEQ ID NO:2, where n and m are integers as described above. Likewise, the invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, which may be described generally as having residues n-m of SEQ ID NO:4, where n and m  
25 are integers as described above.

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers conducted extensive mutational analysis of human cytokine IL-1a (*J. Biol. Chem.* 268:22105-22111 (1993)). They used random mutagenesis to generate over  
30 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]" (see, Abstract). In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide  
35 sequences examined, produced a protein that significantly differed in activity from wild-type.

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Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the N-termini of the respective proteins. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the valine residue at position number 82, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the aspartic acid residue at position number 155, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n'-87 of SEQ ID NO:2, where n' is an integer in the range of 1 to 82, and 83 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues n"-160 of SEQ ID NO:4, where n" is an integer in the range of 1 to 155, and 156 is the position of the first residue from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues R-2 to V-87; V-3 to V-87;

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D-4 to V-87; T-5 to V-87; D-6 to V-87; E-7 to V-87; D-8 to V-87; R-9 to V-87; Y-10 to V-87; P-11 to V-87; Q-12 to V-87; K-13 to V-87; L-14 to V-87; A-15 to V-87; F-16 to V-87; A-17 to V-87; E-18 to V-87; C-19 to V-87; L-20 to V-87; C-21 to V-87; R-22 to V-87; G-23 to V-87; C-24 to V-87; I-25 to V-87; D-26 to V-87; A-27 to V-87; R-28 to V-87; T-29 to V-87; G-30 to V-87; R-31 to V-87; E-32 to V-87; T-33 to V-87; A-34 to V-87; A-35 to V-87; L-36 to V-87; N-37 to V-87; S-38 to V-87; V-39 to V-87; R-40 to V-87; L-41 to V-87; L-42 to V-87; Q-43 to V-87; S-44 to V-87; L-45 to V-87; L-46 to V-87; V-47 to V-87; L-48 to V-87; R-49 to V-87; R-50 to V-87; R-51 to V-87; P-52 to V-87; C-53 to V-87; S-54 to V-87; R-55 to V-87; D-56 to V-87; G-57 to V-87; S-58 to V-87; G-59 to V-87; L-60 to V-87; P-61 to V-87; T-62 to V-87; P-63 to V-87; G-64 to V-87; A-65 to V-87; F-66 to V-87; A-67 to V-87; F-68 to V-87; H-69 to V-87; T-70 to V-87; E-71 to V-87; F-72 to V-87; I-73 to V-87; H-74 to V-87; V-75 to V-87; P-76 to V-87; V-77 to V-87; G-78 to V-87; C-79 to V-87; T-80 to V-87; C-81 to V-87; and V-82 to V-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Further, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues S-2 to P-160; A-3 to P-160; R-4 to P-160; A-5 to P-160; R-6 to P-160; A-7 to P-160; V-8 to P-160; L-9 to P-160; S-10 to P-160; A-11 to P-160; F-12 to P-160; H-13 to P-160; H-14 to P-160; T-15 to P-160; L-16 to P-160; Q-17 to P-160; L-18 to P-160; G-19 to P-160; P-20 to P-160; R-21 to P-160; E-22 to P-160; Q-23 to P-160; A-24 to P-160; R-25 to P-160; N-26 to P-160; A-27 to P-160; S-28 to P-160; C-29 to P-160; P-30 to P-160; A-31 to P-160; G-32 to P-160; G-33 to P-160; R-34 to P-160; P-35 to P-160; A-36 to P-160; D-37 to P-160; R-38 to P-160; R-39 to P-160; F-40 to P-160; R-41 to P-160; P-42 to P-160; P-43 to P-160; T-44 to P-160; N-45 to P-160; L-46 to P-160; R-47 to P-160; S-48 to P-160; V-49 to P-160; S-50 to P-160; P-51 to P-160; W-52 to P-160; A-53 to P-160; Y-54 to P-160; R-55 to P-160; I-56 to P-160; S-57 to P-160; Y-58 to P-160; D-59 to P-160; P-60 to P-160; A-61 to P-160; R-62 to P-160; Y-63 to P-160; P-64 to P-160; R-65 to P-160; Y-66 to P-160; L-67 to P-160; P-68 to P-160; E-69 to P-160; A-70 to P-160; Y-71 to P-160; C-72 to P-160; L-73 to P-160; C-74 to P-160; R-75 to P-160; G-76 to P-160; C-77 to P-160; L-78 to P-160; T-79 to P-160; G-80 to P-160; L-81 to P-160; F-82 to P-160; G-83 to P-160; E-84 to P-160; E-85 to P-160; D-86 to P-160; V-87 to P-160; R-88 to P-160; F-89 to P-160; R-90 to P-160; S-91 to P-160; A-92 to P-160; P-93 to P-160; V-94 to P-160; Y-95 to P-160; M-96 to P-160; P-97 to P-160; T-98 to P-160; V-99 to P-160; V-100 to P-160; L-101 to P-160; R-102 to P-160; R-103 to P-160; T-104 to P-160; P-105 to P-160; A-106 to P-160; C-107 to P-160; A-108 to P-160; G-109 to P-160; G-110 to P-160; R-111 to P-160; S-112 to P-160; V-113 to

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P-160; Y-114 to P-160; T-115 to P-160; E-116 to P-160; A-117 to P-160; Y-118 to P-160; V-119 to P-160; T-120 to P-160; I-121 to P-160; P-122 to P-160; V-123 to P-160; G-124 to P-160; C-125 to P-160; T-126 to P-160; C-127 to P-160; V-128 to P-160; P-129 to P-160; E-130 to P-160; P-131 to P-160; E-132 to P-160; K-133 to  
 5 P-160; D-134 to P-160; A-135 to P-160; D-136 to P-160; S-137 to P-160; I-138 to P-160; N-139 to P-160; S-140 to P-160; S-141 to P-160; I-142 to P-160; D-143 to P-160; K-144 to P-160; Q-145 to P-160; G-146 to P-160; A-147 to P-160; K-148 to P-160; L-149 to P-160; L-150 to P-160; L-151 to P-160; G-152 to P-160; P-153 to P-160; N-154 to P-160; and D-155 to P-160 of SEQ ID NO:4. Polynucleotides  
 10 encoding these polypeptides also are provided.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the  
 15 complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

20 Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the aspartic acid residue at position 6 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed  
 25 from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the arginine residues at position 6 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m' of the amino acid sequence in SEQ ID NO:2, where m' is any integer in the range of 6 to 87, and residue 5 is the position of the first residue from the  
 30 C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. In addition, the present invention also provides polypeptides having the amino acid sequence of residues 1-m" of the amino acid sequence in SEQ ID NO:4, where m" is any integer in the range of 6 to 160, and residue 5 is the position of the first residue from the C-terminus of the  
 35 complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

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More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues A-1 to S-86; A-1 to R-85; A-1 to P-84; A-1 to L-83; A-1 to V-82; A-1 to C-81; A-1 to T-80; A-1 to C-79; A-1 to G-78; A-1 to V-77; A-1 to P-76; A-1 to V-75; A-1 to H-74; A-1 to I-73; A-1 to F-72; A-1 to E-71; A-1 to T-70; A-1 to H-69; A-1 to F-68; A-1 to A-67; A-1 to F-66; A-1 to A-65; A-1 to G-64; A-1 to P-63; A-1 to T-62; A-1 to P-61; A-1 to L-60; A-1 to G-59; A-1 to S-58; A-1 to G-57; A-1 to D-56; A-1 to R-55; A-1 to S-54; A-1 to C-53; A-1 to P-52; A-1 to R-51; A-1 to R-50; A-1 to R-49; A-1 to L-48; A-1 to V-47; A-1 to L-46; A-1 to L-45; A-1 to S-44; A-1 to Q-43; A-1 to L-42; A-1 to L-41; A-1 to R-40; A-1 to V-39; A-1 to S-38; A-1 to N-37; A-1 to L-36; A-1 to A-35; A-1 to A-34; A-1 to T-33; A-1 to E-32; A-1 to R-31; A-1 to G-30; A-1 to T-29; A-1 to R-28; A-1 to A-27; A-1 to D-26; A-1 to I-25; A-1 to C-24; A-1 to G-23; A-1 to R-22; A-1 to C-21; A-1 to L-20; A-1 to C-19; A-1 to E-18; A-1 to A-17; A-1 to F-16; A-1 to A-15; A-1 to L-14; A-1 to K-13; A-1 to Q-12; A-1 to P-11; A-1 to Y-10; A-1 to R-9; A-1 to D-8; A-1 to E-7; and A-1 to D-6 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Moreover, the invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues N-1 to G-159; N-1 to A-158; N-1 to P-157; N-1 to A-156; N-1 to D-155; N-1 to N-154; N-1 to P-153; N-1 to G-152; N-1 to L-151; N-1 to L-150; N-1 to L-149; N-1 to K-148; N-1 to A-147; N-1 to G-146; N-1 to Q-145; N-1 to K-144; N-1 to D-143; N-1 to I-142; N-1 to S-141; N-1 to S-140; N-1 to N-139; N-1 to I-138; N-1 to S-137; N-1 to D-136; N-1 to A-135; N-1 to D-134; N-1 to K-133; N-1 to E-132; N-1 to P-131; N-1 to E-130; N-1 to P-129; N-1 to V-128; N-1 to C-127; N-1 to T-126; N-1 to C-125; N-1 to G-124; N-1 to V-123; N-1 to P-122; N-1 to I-121; N-1 to T-120; N-1 to V-119; N-1 to Y-118; N-1 to A-117; N-1 to E-116; N-1 to T-115; N-1 to Y-114; N-1 to V-113; N-1 to S-112; N-1 to R-111; N-1 to G-110; N-1 to G-109; N-1 to A-108; N-1 to C-107; N-1 to A-106; N-1 to P-105; N-1 to T-104; N-1 to R-103; N-1 to R-102; N-1 to L-101; N-1 to V-100; N-1 to V-99; N-1 to T-98; N-1 to P-97; N-1 to M-96; N-1 to Y-95; N-1 to V-94; N-1 to P-93; N-1 to A-92; N-1 to S-91; N-1 to R-90; N-1 to F-89; N-1 to R-88; N-1 to V-87; N-1 to D-86; N-1 to E-85; N-1 to E-84; N-1 to G-83; N-1 to F-82; N-1 to L-81; N-1 to G-80; N-1 to T-79; N-1 to L-78; N-1 to C-77; N-1 to G-76; N-1 to R-75; N-1 to C-74; N-1 to L-73; N-1 to C-72; N-1 to Y-71; N-1 to A-70; N-1 to E-69; N-1 to P-68; N-1 to L-67; N-1 to Y-66; N-1 to R-65; N-1 to P-64; N-1 to Y-63; N-1 to R-62; N-1 to A-61; N-1 to P-60; N-1 to D-59; N-1 to Y-58; N-1 to S-57; N-1 to I-56; N-1 to R-55; N-1 to Y-54; N-1 to A-53; N-1 to W-52; N-1 to P-51; N-1 to S-50; N-1 to V-49; N-1 to S-48; N-1 to R-47; N-1 to L-46; N-1 to N-45; N-1 to T-44; N-1 to P-43; N-1 to P-42; N-1 to R-41; N-1 to F-40; N-1 to R-39; N-1 to R-38; N-1 to D-37; N-1 to A-36; N-1 to P-35; N-1 to R-34;

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N-1 to G-33; N-1 to G-32; N-1 to A-31; N-1 to P-30; N-1 to C-29; N-1 to S-28; N-1 to A-27; N-1 to N-26; N-1 to R-25; N-1 to A-24; N-1 to Q-23; N-1 to E-22; N-1 to R-21; N-1 to P-20; N-1 to G-19; N-1 to L-18; N-1 to Q-17; N-1 to L-16; N-1 to T-15; N-1 to H-14; N-1 to H-13; N-1 to F-12; N-1 to A-11; N-1 to S-10; N-1 to L-9; N-1 to V-8; N-1 to A-7; and N-1 to R-6 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described generally as having residues n'-m' of SEQ ID NO:2, where n' and m' are integers as described above. Likewise, the invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, which may be described generally as having residues n"-m" of SEQ ID NO:4, where n" and m" are integers as described above.

Moreover, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, described specifically as having residues n"-m" of SEQ ID NO:4 (where n" and m" are integers as described above) may be excluded from the invention. In particular, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22 and which is defined by residues n"-m" of SEQ ID NO:4, where n" is equal to 21, 22, 23, 24 or 25 and m" is equal to 271, 272, 273, 274, 275 or 276 may be excluded from the invention.

The invention further includes IL-21 and IL-22 polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided by Bowie and colleagues (*Science* 247:1306-1310 (1990)), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For

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example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham and Wells, *Science* **244**:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

5 As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains  
10 are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of an aliphatic or hydrophobic amino acid with another aliphatic or hydrophobic amino acid such as Ala, Val, Leu or Ile; replacement of a hydroxyl residue with another hydroxyl residue such as Ser or Thr; replacement of an acidic residue with another acidic residue such as Asp or Glu; replacement of an amide residue with another  
15 amide residue such as Asn or Gln, replacement of a basic residue with another basic residue such as Lys, Arg, or His; replacement of an aromatic residue with another aromatic residue such as Phe, Tyr, or Trp, and replacement of a small-sized amino acid with another small-sized residue such as Ala, Ser, Thr, Met, or Gly.

Besides conservative amino acid substitution, variants of IL-21 and IL-22  
20 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for  
25 example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, IL-21 and IL-22 polypeptide variants containing amino acid  
30 substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (Pinckard, *et al.*, *Clin. Exp. Immunol.* **2**:331-340 (1967); Robbins, *et al.*, *Diabetes* **36**:838-845 (1987); Cleland, *et al.*, *Crit. Rev. Ther.*  
35 *Drug Carrier Systems* **10**:307-377 (1993)).

### Polynucleotide and Polypeptide Fragments

The invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:3 which have been determined from the following related cDNA clones: HE2CD08R (SEQ ID NO:24); HAGBX04R (SEQ ID NO:25); HCEBA24FB (SEQ ID NO:26); and HCELE59R (SEQ ID NO:27).

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clones or shown in SEQ ID NO:1 or SEQ ID NO:3. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in the deposited clones or the nucleotide sequences shown in SEQ ID NO:1 and SEQ ID NO:3. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of IL-21 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, or 701 to the end of SEQ ID NO:1 or the cDNA contained in the deposited clone. In addition, representative examples of IL-22 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1551-1600, or 1601 to the end of SEQ ID NO:3 or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

Further, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 1 to 650, 25 to 650, 50 to 650, 75 to 650, 100 to 650, 125 to 650, 150 to 650, 175 to 650, 200 to 650, 225 to 650, 250 to 650, 275 to 650, 300 to 650, 325 to 650, 350 to 650, 375 to 650, 400 to 650, 425 to 650, 500 to 650, 525 to 650, 550 to 650, 575 to 650, 600 to 650, 625 to 650, 1 to 600, 25 to 600, 50 to 600, 75 to 600, 100 to 600, 125 to 600, 150 to 600, 175 to 600, 200 to 600, 225 to 600, 250 to 600, 275 to 600, 300 to 600, 325 to 600, 350 to 600, 375 to 600, 400 to 600, 425 to 600,

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- 500 to 600, 525 to 600, 550 to 600, 575 to 600, 1 to 550, 25 to 550, 50 to 550, 75 to 550, 100 to 550, 125 to 550, 150 to 550, 175 to 550, 200 to 550, 225 to 550, 250 to 550, 275 to 550, 300 to 550, 325 to 550, 350 to 550, 375 to 550, 400 to 550, 425 to 550, 500 to 550, 525 to 550, 1 to 500, 25 to 500, 50 to 500, 75 to 500, 100 to 500,
- 5 125 to 500, 150 to 500, 175 to 500, 200 to 500, 225 to 500, 250 to 500, 275 to 500, 300 to 500, 325 to 500, 350 to 500, 375 to 500, 400 to 500, 425 to 500, 450 to 500, 475 to 500, 1 to 450, 25 to 450, 50 to 450, 75 to 450, 100 to 450, 125 to 450, 150 to 450, 175 to 450, 200 to 450, 225 to 450, 250 to 450, 275 to 450, 300 to 450, 325 to 450, 350 to 450, 375 to 450, 400 to 450, 425 to 450, 1 to 400, 25 to 400, 50 to 400,
- 10 75 to 400, 100 to 400, 125 to 400, 150 to 400, 175 to 400, 200 to 400, 225 to 400, 250 to 400, 275 to 400, 300 to 400, 325 to 400, 350 to 400, 375 to 400, 1 to 350, 25 to 350, 50 to 350, 75 to 350, 100 to 350, 125 to 350, 150 to 350, 175 to 350, 200 to 350, 225 to 350, 250 to 350, 275 to 350, 300 to 350, 325 to 350, 1 to 300, 25 to 300, 50 to 300, 75 to 300, 100 to 300, 125 to 300, 150 to 300, 175 to 300, 200 to 300, 225 to 300, 250 to 300, 275 to 300, 1 to 250, 25 to 250, 50 to 250, 75 to 250, 100 to 250,
- 15 125 to 250, 150 to 250, 175 to 250, 200 to 250, 225 to 250, 1 to 200, 25 to 200, 50 to 200, 75 to 200, 100 to 200, 125 to 200, 150 to 200, 175 to 200, 1 to 150, 25 to 150, 50 to 150, 75 to 150, 100 to 150, 125 to 150, 1 to 100, 25 to 100, 50 to 100, 75 to 100, 1 to 50, and 25 to 50.
- 20 Moreover, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:3 from residue 300 to 850. More preferably, the invention includes a polynucleotide comprising nucleotide residues 50 to 850, 75 to 850, 100 to 850, 125 to 850, 150 to 850, 175 to 850, 200 to 850, 225 to 850, 250 to 850, 275 to 850, 300 to 850, 325 to 850, 350 to 850, 375 to 850, 400 to 850, 425 to 850, 450 to 850, 475 to 850, 500 to 850, 525 to 850, 550 to 850, 575 to 850, 600 to 850, 625 to 850, 650 to 850, 675 to 850, 700 to 850, 750 to 850, 775 to 850, 800 to 850, 50 to 800, 75 to 800, 100 to 800, 125 to 800, 150 to 800, 175 to 800, 200 to 800, 225 to 800, 250 to 800, 275 to 800, 300 to 800, 325 to 800, 350 to 800, 375 to 800, 400 to 800, 425 to 800, 450 to 800, 475 to 800, 500 to 800, 525 to 800, 550 to 800, 575 to 800, 600 to 800, 625 to 800, 650 to 800, 675 to 800, 700 to 800, 750 to 800, 50 to 750, 75 to 750, 100 to 750, 125 to 750, 150 to 750, 175 to 750, 200 to 750, 225 to 750, 250 to 750, 275 to 750, 300 to 750, 325 to 750, 350 to 750, 375 to 750, 400 to 750, 425 to 750, 450 to 750, 475 to 750, 500 to 750, 525 to 750, 550 to 750, 575 to 750, 600 to 750, 625 to 750, 650 to 750, 675 to 750, 700 to 750, 50 to 700, 75 to 700, 100 to 700, 125 to 700, 150 to 700, 175 to 700, 200 to 700, 225 to 700, 250 to 700, 275 to 700, 300 to 700, 325 to 700, 350 to 700, 375 to 700, 400 to 700, 425 to 700, 450 to 700, 475 to 700,
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In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:2 or SEQ ID NO:4 or encoded by the cDNAs contained in the deposited clones. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the IL-21 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-83 or to the end of the coding region. Moreover, polypeptide fragments of IL-21 can be about 10, 20, 30, 40, 50, 60, 70, or 80 amino acids in length. Representative examples of polypeptide fragments of the IL-22 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 100-120, 120-140, 140-160, or to the end of the coding region. Moreover, polypeptide fragments of IL-22 can be about 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted IL-21 and IL-22 proteins as well as the mature forms. Further preferred polypeptide fragments include the

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secreted IL-21 and IL-22 proteins or the mature forms having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted or the mature form of the IL-21 and IL-22 polypeptides. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted or the mature form of the IL-21 and IL-22 polypeptides. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these IL-21 and IL-22 polypeptide fragments are also preferred.

Also preferred are IL-21 and IL-22 polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:2 and SEQ ID NO:4 falling within conserved domains are specifically contemplated by the present invention (Figures 4 and 5, respectively). Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active IL-21 and IL-22 fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the IL-21 and IL-22 polypeptides. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

#### Epitopes & Antibodies

In the present invention, "epitopes" refer to IL-21 and IL-22 polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to an IL-21 or IL-22 polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope". In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response (see, for instance, Geysen, *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998- 4002 (1983)).

Fragments which function as epitopes may be produced by any conventional means (see, e.g., Houghten, R. A., *Proc. Natl. Acad. Sci. USA* 82:5131-5135 (1985); further described in U.S. Patent No. 4,631,211).

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In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope (see, for instance, Wilson, *et al.*, *Cell* 37:767-778 (1984); Sutcliffe, J. G. *et al.*, *Science* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (see, for instance, Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*; Chow, M., *et al.*, *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F. J., *et al.*, *J. Gen. Virol.* 66:2347-2354 (1985)). A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Using DNASTar analysis, SEQ ID NO:2 was found antigenic at amino acids: from about Arg-2 to about Pro-11, from about Cys-24 to about Glu-32, and from about Arg-51 to about Gly-59. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HTGED19. Again using DNASTar analysis, SEQ ID NO:4 was found antigenic at amino acids: from about Gly-19 to about Ala-27, from about Pro-30 to about Arg-38, from about Phe-40 to about Ser-48, from about Tyr-58 to about Leu-67, from about Pro-105 to about Val-113, from about Pro-129 to about Ser-137, from about Asn-139 to about Ala-147, and from about Leu-151 to about Gly-159. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HFPBX96.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to protein. Fab and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl, *et al.*, *J. Nucl. Med.* 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

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### Fusion Proteins

Any IL-21 or IL-22 polypeptide can be used to generate fusion proteins. For example, the IL-21 or IL-22 polypeptides, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the IL-21 or IL-22 polypeptides can be used to indirectly detect a second protein by binding to IL-21 or IL-22, respectively. Moreover, because secreted proteins target cellular locations based on trafficking signals, the IL-21 and IL-22 polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to the IL-21 and IL-22 polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the IL-21 and IL-22 polypeptides. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the IL-21 and IL-22 polypeptides to improve stability and persistence during purification from the host cell or during subsequent handling and storage. Also, peptide moieties may be added to the IL-21 and IL-22 polypeptides to facilitate purification. Such regions may be removed prior to final preparation of the IL-21 and IL-22 polypeptides. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, IL-21 and IL-22 polypeptides, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker, *et al.*, *Nature* 331:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (Fountoulakis, *et al.*, *J. Biochem.* 270:3958-3964 (1995)).

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A 0232 262). Alternatively,

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deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (see; Bennett, D., *et al.*, *J. Mol. Recog.* 8:52-58 (1995); Johanson, K., *et al.*, *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the IL-21 and IL-22 polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of IL-21 and IL-22, respectively. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described by Gentz and coworkers (*Proc. Natl. Acad. Sci. USA* 86:821-824 (1989)), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, *et al.*, *Cell* 37:767 (1984)).

Thus, any of the above fusion proteins can be engineered using the IL-21 and IL-22 polynucleotides or the polypeptides.

#### Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the IL-21 and IL-22 polynucleotides, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

IL-21 and IL-22 polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The IL-21 and IL-22 polynucleotide inserts should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding

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portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one  
 5 selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium* cells; fungal cells, such as yeast cells;  
 10 insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pHE4-5 and other pHE-like vectors; pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript  
 15 vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWL.NEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be  
 20 readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals (for example, Davis, *et al.*, *Basic*  
 25 *Methods In Molecular Biology* (1986)). It is specifically contemplated that IL-21 and IL-22 polypeptides may, in fact, be expressed by a host cell lacking a recombinant vector.

IL-21 and IL-22 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol  
 30 precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

35 IL-21 and IL-22 polypeptides, and preferably the secreted forms thereof, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical

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synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the IL-21 and IL-22 polypeptides may be glycosylated or may be non-glycosylated. In addition, IL-21 and IL-22 polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

#### Uses of the IL-21 and IL-22 Polynucleotides

The IL-21 and IL-22 polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Clone HTGED19 and clone HFPBX96 can each be mapped to a specific chromosome. Thus, IL-21 and IL-22 polynucleotides can then be used in linkage analysis as a marker for those specific chromosome.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1 and SEQ ID NO:3. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human IL-21 or IL-22 genes corresponding to SEQ ID NO:1 or SEQ ID NO:3, respectively, will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the IL-21 and IL-22 polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

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Precise chromosomal location of the IL-21 and IL-22 polynucleotides can also be achieved using fluorescence *in situ* hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred (For review, see Verma, *et al.*, "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988)).

For chromosome mapping, the IL-21 and IL-22 polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease (disease mapping data are found, for example, in McKusick, V., Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the IL-21 and IL-22 polynucleotides and the corresponding genes between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the IL-21 and IL-22 polypeptides and the corresponding genes from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using IL-21 and IL-22 polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, an IL-21 or IL-22 polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both

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methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee, *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney, *et al.*, *Science* 241:456 (1988); and Dervan, *et al.*, *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. *Neurochem.* 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

IL-21 and IL-22 polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. IL-21 and IL-22 offer means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The IL-21 and IL-22 polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The IL-21 and IL-22 polynucleotides can be used as additional DNA markers for RFLP.

The IL-21 and IL-22 polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from

polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals (Erich, H., *PCR Technology*, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, IL-21 and IL-22 polynucleotides can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from IL-21 and IL-22 sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

Because IL-21 is found expressed almost exclusively in apoptotic T-cells, IL-21 polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-21 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-21 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-21 gene expression level, i.e., the IL-21 expression level in healthy tissue from an individual not having the Immune system disorder.

Likewise, since IL-22 is found expressed in bone marrow, skeletal muscle, and brain, IL-22 polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-22 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-22 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-22 gene expression level, i.e., the IL-22 expression level in healthy tissue from an individual not having the Immune system disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves:  
(a) assaying IL-21 or IL-22 gene expression level in cells or body fluid of an individual;

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(b) comparing the IL-21 or IL-22 gene expression level with a standard IL-21 or IL-22 gene expression level, respectively, whereby an increase or decrease in the assayed IL-21 or IL-22 gene expression level compared to the standard expression level is indicative of disorder in the Immune system.

- 5 In the very least, the IL-21 and IL-22 polynucleotides can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA  
10 immunization techniques, and as an antigen to elicit an immune response.

#### Uses of IL-21 and IL-22 Polypeptides

IL-21 and IL-22 polypeptides can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

- 15 IL-21 and IL-22 polypeptides can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* **101**:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell. Biol.* **105**:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression  
20 include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

- 25 In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for  
30 NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

- A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  
35  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the

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subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99m}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially  
 5 accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described by Burchiel and colleagues ("Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

10 Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of IL-21 or IL-22 polypeptides in cells or body fluid of an individual; (b) comparing the level of IL-21 or IL-22 gene expression with a standard gene expression level, whereby an increase or decrease in the assayed IL-21 or IL-22 polypeptide gene expression level compared to the standard expression level is  
 15 indicative of a disorder.

Moreover, IL-21 and IL-22 polypeptides can be used to treat disease. For example, patients can be administered IL-21 and IL-22 polypeptides in an effort to replace absent or decreased levels of the IL-21 and IL-22 polypeptides, respectively, (e.g., insulin), to supplement absent or decreased levels of a different polypeptide  
 20 (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

25 Similarly, antibodies directed to IL-21 and IL-22 polypeptides can also be used to treat disease. For example, administration of an antibody directed to an IL-21 or IL-22 polypeptide can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

30 At the very least, the IL-21 and IL-22 polypeptides can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. IL-21 and IL-22 polypeptides can also be used to raise antibodies, which, in turn, are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover,

35 IL-21 and IL-22 polypeptides can be used to test the following biological activities.

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### Biological Activities of IL-21 and IL-22

IL-21 and IL-22 polynucleotides and polypeptides can be used in assays to test for one or more biological activities. If IL-21 and IL-22 polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that IL-21 and IL-22 may be involved in the diseases associated with the biological activity. Therefore, IL-21 and IL-22 could be used to treat the associated disease.

The IL-21 and IL-22 proteins of the present invention modulate IL-6 secretion from NIH-3T3 cells. An *in vitro* ELISA assay which quantitates the amount of IL-6 secreted from cells in response to treatment with cytokines or the soluble extracellular domains of cytokine receptors has been described (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Briefly, the assay involves plating the target cells at a density of approximately  $5 \times 10^6$  cells/mL in a volume of 500  $\mu$ L in the wells of a 24 well flat-bottomed culture plate (Costar). The cultures are then treated with various concentrations of the cytokine or the soluble extracellular domain of cytokine receptor in question. The cells are then cultured for 24 hours at 37°C. At this time, 50  $\mu$ L of supernatant is removed and assayed for the quantity of IL-6 essentially as described by the manufacturer (Genzyme, Boston, MA). IL-6 levels are then calculated by reference to a standard curve constructed with recombinant IL-17 cytokine. Such activity is useful for determining the level of IL-21- or IL-22-mediated IL-6 secretion.

IL-21 and IL-22 protein modulates immune system cell proliferation and differentiation in a dose-dependent manner in the above-described assay. Thus, "a polypeptide having IL-21 or IL-22 protein activity" includes polypeptides that also exhibit any of the same stimulatory activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the IL-21 or IL-22 proteins, preferably, "a polypeptide having IL-21 or IL-22 protein activity" will exhibit substantially similar dose-dependence in a given activity as compared to the IL-21 or IL-22 protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference IL-21 or IL-22 protein).

Lymphocyte proliferation is another *in vitro* assay which may be performed to determine the activity of IL-21 and IL-22. For example, Yao and colleagues (*Immunity* 3:811-821 (1995)) have recently described an *in vitro* assay for determining the effects of various cytokines and soluble cytokine receptors on the proliferation of murine leukocytes. Briefly, lymphoid organs are harvested aseptically, lymphocytes are isolated from the harvested organs, and the resulting collection of lymphoid cells are suspended in standard culture medium as described by Fanslow and coworkers (*J. Immunol.* 147:535-5540 (1991)). The lymphoid cell suspensions may then be divided

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into several different subclasses of lymphoid cells including splenic T-cells, lymph node B-cells, CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, and mature adult thymocytes. For splenic T-cells, spleen cell suspensions ( $200 \times 10^6$  cells) are incubated with CD11b mAb and class II MHC mAb for 30 min at 4°C, loaded on a T-cell purification column (Pierce, Rockford, IL), and the T-cells eluted according to the manufacturer's instructions. Using this method, purity of the resulting T-cell populations should be >95% CD3<sup>+</sup> and <1% sIgM<sup>+</sup>. For purification of lymph node subsets, B-cells are removed from by adherence to tissue culture dishes previously coated with goat anti-mouse IgG (10 µg/mL). Remaining cells were then incubated with anti-CD4 or anti-CD8 for 30 min at 4°C then washed and placed on tissue culture dishes previously coated with goat anti-rat IgG (20 µg/mL). After 45 min, nonadherent cells are removed and tested for purity by flow cytometry. CD4 and surface Ig-depleted cells should be >90% TCR-ab, CD8<sup>+</sup>, whereas CD8 and surface Ig-depleted cells should be >95% TCR-ab, CD4<sup>+</sup>. Finally, to enrich for mature adult thymocytes, cells are suspended at  $10^8$ /mL in 10% anti-HSA and 10% low tox rabbit complement (Cedarlane, Ontario, Canada), incubated for 45 min at 37°C, and remaining viable cells isolated over Ficoll-Hypaque (Pharmacia, Piscataway, NJ). This procedure should yield between 90 and 95% CD3<sup>hi</sup> cells that are either CD4<sup>+</sup> or CD8<sup>+</sup>.

#### 20 Immune Activity

IL-21 and IL-22 polypeptides or polynucleotides may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, IL-21 and IL-22 polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

IL-21 and IL-22 polynucleotides or polypeptides may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. IL-21 and IL-22 polypeptides or polynucleotides could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia

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telangiectasia, common variable immunodeficiency, DiGeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, IL-21 and IL-22 polypeptides or polynucleotides can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, IL-21 and IL-22 polynucleotides or polypeptides could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, IL-21 and IL-22 polynucleotides or polypeptides that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting, important in the treatment of heart attacks (infarction), strokes, or scarring.

IL-21 and IL-22 polynucleotides or polypeptides may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of IL-21 and IL-22 polypeptides or polynucleotides that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by IL-21 and IL-22 include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by IL-21 and IL-22 polypeptides or polynucleotides. Moreover, IL-21 and IL-22 can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

IL-21 and IL-22 polynucleotides or polypeptides may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune

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response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of IL-21 and IL-22 polypeptides or polynucleotides that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate inflammation. For example, IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### Hyperproliferative Disorders

IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect hyperproliferative disorders, including neoplasms. IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, IL-21 and IL-22 polypeptides or polynucleotides may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of such hyperproliferative

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disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

#### **Infectious Disease**

IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, IL-21 and IL-22 polypeptides or polynucleotides may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia),

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- Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis,
- 5 Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Pasteurellaceae Infections (e.g., Actinobacillus, Haemophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections
- 10 (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria,
- 15 Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.
- 20 Moreover, parasitic agents causing disease or symptoms that can be treated or detected by IL-21 polynucleotides or polypeptides include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can
- 25 cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.
- 30 Preferably, treatment using IL-21 and IL-22 polypeptides or polynucleotides could either be by administering an effective amount of IL-21 or IL-22 polypeptide to the patient, or by removing cells from the patient, supplying the cells with IL-21 and IL-22 polynucleotide, and returning the engineered cells to the patient (*ex vivo* therapy). Moreover, the IL-21 and IL-22 polypeptide or polynucleotide can be used as an antigen
- 35 in a vaccine to raise an immune response against infectious disease.

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### Regeneration

IL-21 and IL-22 polynucleotides or polypeptides can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (see, *Science* 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, IL-21 and IL-22 polynucleotides or polypeptides may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. IL-21 and IL-22 polynucleotides or polypeptides of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using IL-21 and IL-22 polynucleotides or polypeptides to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the IL-21 and IL-22 polynucleotides or polypeptides.

### Chemotaxis

IL-21 and IL-22 polynucleotides or polypeptides may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a

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particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

IL-21 and IL-22 polynucleotides or polypeptides may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, IL-21 and IL-22 could also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that IL-21 and IL-22 polynucleotides or polypeptides may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, IL-21 and IL-22 polynucleotides or polypeptides could be used as an inhibitor of chemotaxis.

#### **Binding Activity**

IL-21 and IL-22 polypeptides may be used to screen for molecules that bind to IL-21 or IL-22 or for molecules to which IL-21 or IL-22 bind. The binding of IL-21 and IL-22 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the IL-21 and IL-22 or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of IL-21 or IL-22, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (see, Coligan, *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which IL-21 and IL-22 bind, or at least, a fragment of the receptor capable of being bound by IL-21 or IL-22 (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express IL-21 and IL-22, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing IL-21 and IL-22 (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either IL-21 and IL-22 or the molecule.

The assay may simply test binding of a candidate compound to IL-21 or IL-22, wherein binding is detected by a label, or in an assay involving competition with a



labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to IL-21 or IL-22.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing IL-21 or IL-22, measuring IL-21/molecule or IL-22/molecule activity or binding, respectively, and comparing the IL-21/molecule or IL-22/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure IL-21 and IL-22 levels or activities in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure IL-21 and IL-22 levels or activities by either binding, directly or indirectly, to IL-21 or IL-22 or by competing with IL-21 or IL-22 for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting IL-21 or IL-22. Moreover, the assays can discover agents which may inhibit or enhance the production of IL-21 and IL-22 from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to IL-21 and IL-22 comprising the steps of: (a) incubating a candidate binding compound with IL-21 or IL-22; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with IL-21 or IL-22, (b) assaying a biological activity, and (c) determining if a biological activity of IL-21 or IL-22, respectively, has been altered.

#### Other Activities

IL-21 and IL-22 polypeptides or polynucleotides may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, IL-21 and IL-22 polypeptides or polynucleotides may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

IL-21 and IL-22 polypeptides or polynucleotides may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms,

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depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

IL-21 and IL-22 polypeptides or polynucleotides may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

#### Examples

In the case where IL-22 is not specifically mentioned, specific details are provided in the following examples only for the IL-21 molecules of the present invention. However, the examples can also be easily performed for the IL-22 molecules of the present invention by using the details provided for IL-21 and substituting appropriate nucleotides or amino acid residues of IL-22, for example, in the design of suitable PCR primers, and the like. The use or applicability of IL-22 in place of IL-21 is thus contemplated in each of the following examples. When provided with the nucleotide and amino acid sequences of IL-21 (SEQ ID NO:1 and SEQ ID NO:2) and IL-22 (SEQ ID NO:3 and SEQ ID NO:4) of the present invention, one of ordinary skill in the art could easily perform the following examples with the intent of isolating or further characterizing or manipulating IL-22 in place of IL-21.

#### Example 1: Isolation of the IL-21 and IL-22 cDNA Clones From the Deposited Samples

The cDNAs encoding IL-21 and IL-22 are each inserted into the *Eco* RI and *Xho* I restriction sites of the multiple cloning site of pBluescript. pBluescript contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies (see, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993)).

Two approaches can be used to isolate IL-21 from the deposited sample. First, a specific polynucleotide of SEQ ID NO:1 with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods (e.g., Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982)). The

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plasmid mixture is transformed into a suitable host (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 (i.e., within the region of SEQ ID NO:1 bounded by the 5' and 3' nucleotides of the clone) are synthesized and used to amplify the IL-21 cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5  $\mu$ g of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $MgCl_2$ , 0.01% (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of the IL-21 gene which may not be present in the deposited clone. These methods include, but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' RACE protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript (Fromont-Racine, *et al.*, *Nucl. Acids Res.* 21(7):1683-1684 (1993)).

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the IL-21 gene of interest is used to PCR amplify the 5' portion of the IL-21 full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with a

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phosphatase, if necessary, to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNA. This reaction leaves  
 5 a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to  
 10 the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the IL-21 gene.

#### **Example 2: Isolation of IL-21 Genomic Clones**

15 A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:1., according to the method described in Example 1 (see also, Sambrook, *et al.*, *supra*).

#### **Example 3: Tissue Distribution of IL-21**

20 Tissue distribution of mRNA expression of IL-21 is determined using protocols for Northern blot analysis, described by, among others, Sambrook and colleagues (*supra*). For example, an IL-21 probe produced by the method described in Example 1 is labeled with <sup>32</sup>P using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified  
 25 using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system (IM) tissues (Clontech) are examined with the labeled probe  
 30 using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

Using essentially the above-prescribed protocol, Northern blot analyses were  
 35 performed to determine the expression pattern of IL-21 and IL-22. In the case of IL-21, a major message of approximately 5 kb was detected predominantly in thymus, but was also detectable in adrenal cortex, spleen, pancreas, and very weakly in lymph

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node, PBL, fetal liver, adrenal medulla, thyroid, small intestine, stomach, and heart. In the case of IL-22, a major message of slightly less than 1 kb was detected in conjunction with a minor band of approximately 5 kb predominantly in testis and spinal cord, but was also detected in bone marrow and small intestine.

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#### Example 4: Chromosomal Mapping of IL-21

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

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#### Example 5: Bacterial Expression of IL-21

An IL-21 polynucleotide encoding an IL-21 polypeptide of the invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as *Bam* HI and *Hin* dIII, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, *Bam* HI and *Hin* dIII correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>R</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

25

Specifically, to clone the mature domain of the IL-21 protein in a bacterial vector, the 5' primer has the sequence 5'-GAT CGC GGA TCC GAC ACG GAT GAG GAC CGC TAT CCA CAG AAG CTG-3' (SEQ ID NO:9) containing the underlined *Bam* HI restriction site followed several nucleotides of the amino terminal coding sequence of the mature IL-21 sequence in SEQ ID NO:1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the 5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete IL-21 protein shorter or longer than the mature form of the

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protein. The 3' primer has the sequence 5'-CCC AAG CTT TCA CAC TGA ACG GGG CAG CAC GCA GGT GCA GC-3' (SEQ ID NO:10) containing the underlined *Hin* dIII restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of the IL-21 DNA sequence of SEQ ID NO:1.

- 5 The pQE-9 vector is digested with *Bam* HI and *Hin* dIII and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>).
- 10 Transformants are identified by their ability to grow on LB plates and colonies are selected which are resistant to both ampicillin and kanamycin. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml).

- 15 The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sub>600</sub>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the promoter/operator leading to increased gene expression.
- 20 Cells are grown for an additional 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000 X g). The cell pellet is solubilized in the chaotropic agent 6 M Guanidine-HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (QIAGEN, Inc., *supra*).
- 25 Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

- 30 Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

- 35 The purified IL-21 protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the IL-21 protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of

1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified IL-21 protein is stored at 4°C or frozen at -80°C.

5 In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to an IL-21 polynucleotide, called pHE4a (ATCC Accession Number 209645, deposited February 25, 1998). This vector contains: (1) a neomycin phosphotransferase gene as a selection marker, (2) an *E. coli* origin of replication, (3) a  
10 T5 phage promoter sequence, (4) two lac operator sequences, (5) a Shine-Delgarno sequence, and (6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LT1, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with *Nde* I and *Xba* I,  
15 I, *Bam* HI, *Xho* I, or *Asp* 718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers which encode restriction sites for *Nde* I (5' primer) and *Nde* I and *Xba* I, *Bam* HI, *Xho* I, or *Asp* 718 (3' primer). The PCR insert is gel purified and restricted with compatible  
20 enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### **Example 6: Purification of IL-21 Polypeptide from an Inclusion Body**

25 The following alternative method can be used to purify IL-21 polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at  
30 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

35 The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by

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centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x g centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 x g) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the IL-21 polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant IL-21 polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 µg of purified protein is loaded. The purified IL-21 protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

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**Example 7: Cloning and Expression of IL-21 in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert IL-21 polynucleotide into a baculovirus to express IL-21. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned IL-21 polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, by Luckow and colleagues (*Virology* 170:31-39 (1989)).

Specifically, the IL-21 cDNA sequence contained in the deposited clone, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. However, since the predicted naturally occurring signal peptides of IL-21 and IL-22 are not known, the vector can be modified (now designated pA2GP) to include a baculovirus leader sequence, using the standard methods described by Summers and coworkers ("A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987)).

More specifically, the cDNA sequence encoding the full-length IL-21 protein in the deposited clone is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-CGC CGC GGA TCC GCC ATC CGC ACG AGT GGA CAC GG-3' (SEQ ID NO:11) containing the *Bam* HI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (shown in the primer sequence in italics; Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), a "C" residue to preserve the reading frame, and 16 nucleotides of the sequence of the complete IL-21 protein shown in Figure 1. The 3' primer has the

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sequence 5'-CGC GGT ACC CAC TGA ACG GGG CAG CAC GC-3' (SEQ ID NO:12) containing the *Asp* 718 restriction site followed by 20 nucleotides complementary to the 3' noncoding sequence in Figure 1.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, CA). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, CA).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five µg of a plasmid containing the polynucleotide is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner and colleagues (*Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987)). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith (*supra*). An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.)

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After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 5 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection 10 ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The 15 proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced IL-21 20 protein.

#### Example 8: Expression of IL-21 in Mammalian Cells

IL-21 polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the 25 termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV-I, HIV-1 and the early promoter of the 30 cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), 35 pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

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Alternatively, IL-21 polypeptide can be expressed in stable cell lines containing the IL-21 polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as *dhfr*, *gpt*, neomycin or hygromycin allows the identification and isolation of the transfected cells.

5 The transfected IL-21 gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest (see, e.g., Alt, F. W., *et al.*, *J. Biol. Chem.* **253**:1357-1370 (1978); Hamlin, J. L. and Ma, C., *Biochem. et Biophys. Acta*, **1097**:107-143 (1990); Page, M. J. and Sydenham, M. A., *Biotechnology* **9**:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS; Murphy, *et al.*, *Biochem. J.* **227**:277-279 (1991); Bebbington, *et al.*, *BioTechnology* **10**:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a  
10 chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, *et al.*, *Mol. Cell. Biol.*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart, *et al.*, *Cell* **41**:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of IL-21. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the  
15 SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

IL-21 polynucleotide is amplified according to the protocol outlined in Example  
20 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

The amplified fragment is isolated from a 1% agarose gel using a commercially  
35 available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

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The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner, et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (for example, 50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200  $\mu$ M. Expression of IL-21 is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### **Example 9: Protein Fusions of IL-21**

IL-21 polypeptides are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of IL-21 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification (see Example 5; see also EP A 394,827; Traunecker, et al., *Nature* 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time *in vivo*. Nuclear localization signals fused to IL-21 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

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Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

5 For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the *Bam* HI cloning site. Note that the 3' *Bam* HI site should be destroyed. Next, the vector containing the human Fc portion is again restricted with *Bam* HI, linearizing the vector, and IL-21 polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this *Bam* HI site. Note that the polynucleotide is  
10 cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

15 **Human IgG Fc region (SEQ ID NO:13):**

GGGATCGGAGCCCAATCTTCTGACAAAACCTCACACATGCCACCGTGCCTAGCACCTGAATTGAGGGTGCACCGTCAGTC  
TTCTCTTCTCCCAAAACCCCAAGGACACCTCATGATCTCCCGGACTCTGAGGTGCATGCGTGGTGGTGAAGTAAAGCCA  
CGAAGACCCCTGAGGTCAAGTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCCGGGAGGAGCAGT  
20 ACAACAGCAGCTACCGTGTGGTACGGTCTCCACCGTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC  
TCCAAAGAGCCCTCCCAACCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTT  
GCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTACAGCTGACCTGCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCG  
TGGAGTGGGAGAGCAATGGGAGCCGAGAGCAACTACAAGACAGGCTCCCGTGTGGACTCCGACGGCTCTTCTTCTCTC  
TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTCTCTCATGCTCCGTGATGATGAGGCTCTGCACAA  
25 CCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGGACGGCCGCGACTCTAGAGGAT

#### **Example 10: Production of an Antibody**

The antibodies of the present invention can be prepared by a variety of methods (see, Current Protocols, Chapter 2). For example, cells expressing IL-21 is  
30 administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of IL-21 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

35 In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al.*, *Nature*

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256:495 (1975); Kohler, *et al.*, *Eur. J. Immunol.* 6:511 (1976); Kohler, *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with IL-21 polypeptide or, more  
5 preferably, with a secreted IL-21 polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

10 The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as  
15 described by Wands and colleagues (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the IL-21 polypeptide.

Alternatively, additional antibodies capable of binding to IL-21 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method  
20 makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody  
25 whose ability to bind to the IL-21 protein-specific antibody can be blocked by IL-21. Such antibodies comprise anti-idiotypic antibodies to the IL-21 protein-specific antibody and can be used to immunize an animal to induce formation of further IL-21 protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies  
30 of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted IL-21 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

35 For *in vivo* use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies

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described above. Methods for producing chimeric antibodies are known in the art (see, for review, Morrison, *Science* **229**:1202 (1985); Oi, *et al.*, *BioTechniques* **4**:214 (1986); Cabilly, *et al.*, U.S. Patent No. 4,816,567; Taniguchi, *et al.*, EP 171496; Morrison, *et al.*, EP 173494; Neuberger, *et al.*, WO 8601533; Robinson, *et al.*, WO 8702671; Boulianne, *et al.*, *Nature* **312**:643 (1984); Neuberger, *et al.*, *Nature* **314**:268 (1985)).

**Example 11: Production Of IL-21 Protein For High-Throughput Screening Assays**

10 The following protocol produces a supernatant containing IL-21 polypeptide to be tested. This supernatant can then be used in the screening assays described subsequently in Examples 13-20.

First, dilute poly-D-lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (Phosphate Buffered Saline; w/o calcium or magnesium  
15 17-516F Biowhittaker) for a working solution of 50 µg/ml. Add 200 µl of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate the poly-D-lysine solution and rinse with 1 ml PBS. The PBS should remain in the well until just prior to plating the cells and plates may be  
20 poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in 0.5 ml DMEM (Dulbecco's Modified Eagle Medium) supplemented with 4.5 G/L glucose, L-glutamine (12-604F Biowhittaker), 10% heat inactivated FBS (14-503F Biowhittaker), and 1x Penstrep (17-602E Biowhittaker). Let the cells grow overnight.

25 Following overnight incubation, mix together in a sterile solution basin: 300 µl Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL) in each well of a 96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2 µg of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled  
30 96-well round bottom plate. With a multi-channel pipetter, add 50 µl of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT for 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150 µl Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

35 Preferably, the transfection should be performed by simultaneously performing the following tasks in a staggered fashion. Thus, hands-on time is cut in half, and the

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cells are not excessively incubated in PBS. First, person A aspirates the media from four 24-well plates of cells, and then person B rinses each well with 0.5-1ml PBS. Person A then aspirates the PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200  $\mu$ l of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Plates are then incubated at 37°C for 6 hours.

While cells are incubating, the appropriate media is prepared: either 1% BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; 4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; 0.070 mg/L of D-L-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L-Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na- $2\text{H}_2\text{O}$ ; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolality to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50  $\mu$ l for endotoxin assay in 15ml polystyrene conical.

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The transfection reaction is terminated, again, preferably by two people, at the end of the incubation period. Person A aspirates the transfection media, while person B adds 1.5 ml of the appropriate media to each well. Incubate at 37°C for 45 or 72 hours, depending on the media used (1%BSA for 45 hours or CHO-5 for 72 hours).

5 On day four, using a 300 µl multichannel pipetter, aliquot 600 µl in one 1ml deep well plate and the remaining supernatant into a 2 ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the IL-21  
10 polypeptide directly (e.g., as a secreted protein) or by IL-21 inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### 15 **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site ("GAS") elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a  
20 protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in  
25 many cell types though it has been found in T-helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon  
30 tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table  
35 below (adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995)). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11,

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IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (where  
 5 "Xxx" represents any amino acid; SEQ ID NO:14)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of  
 10 the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (see Table below). Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

|    | ISRE<br>Ligand       | JAKs |      |      |      | STATs | GAS(elements) or |
|----|----------------------|------|------|------|------|-------|------------------|
|    |                      | tyk2 | Jak1 | Jak2 | Jak3 |       |                  |
| 20 | <u>IFN family</u>    |      |      |      |      |       |                  |
|    | IFN- $\alpha$ /B     | +    | +    | -    | -    | 1,2,3 | ISRE             |
|    | IFN- $\gamma$        |      | +    | +    | -    | 1     | GAS              |
|    | (IRF1>Lys6>IFP)      |      |      |      |      |       |                  |
|    | IL-10                | +    | ?    | ?    | -    | 1,3   |                  |
| 25 | <u>gp130 family</u>  |      |      |      |      |       |                  |
|    | IL-6 (Pleiotrohic)   | +    | +    | +    | ?    | 1,3   | GAS              |
|    | (IRF1>Lys6>IFP)      |      |      |      |      |       |                  |
|    | IL-11(Pleiotrohic)   | ?    | +    | ?    | ?    | 1,3   |                  |
|    | OnM(Pleiotrohic)     | ?    | +    | +    | ?    | 1,3   |                  |
|    | LIF(Pleiotrohic)     | ?    | +    | +    | ?    | 1,3   |                  |
|    | CNTF(Pleiotrohic)    | -/+  | +    | +    | ?    | 1,3   |                  |
|    | G-CSF(Pleiotrohic)   | ?    | +    | ?    | ?    | 1,3   |                  |
|    | IL-12(Pleiotrohic)   | +    | -    | +    | +    | 1,3   |                  |
| 35 | <u>g-C family</u>    |      |      |      |      |       |                  |
|    | IL-2 (lymphocytes)   | -    | +    | -    | +    | 1,3,5 | GAS              |
|    | IL-4 (lymph/myeloid) | -    | +    | -    | +    | 6     | GAS (IRF1 = IFP  |
|    | >>Ly6(IgH)           |      |      |      |      |       |                  |
|    | IL-7 (lymphocytes)   | -    | +    | -    | +    | 5     | GAS              |
|    | IL-9 (lymphocytes)   | -    | +    | -    | +    | 5     | GAS              |
|    | IL-13 (lymphocyte)   | -    | +    | ?    | ?    | 6     | GAS              |
|    | IL-15                | ?    | +    | ?    | +    | 5     | GAS              |
| 45 | <u>gp140 family</u>  |      |      |      |      |       |                  |
|    | IL-3 (myeloid)       | -    | -    | +    | -    | 5     | GAS              |
|    | (IRF1>IFP>>Ly6)      |      |      |      |      |       |                  |
|    | IL-5 (myeloid)       | -    | -    | +    | -    | 5     | GAS              |
|    | GM-CSF (myeloid)     | -    | -    | +    | -    | 5     | GAS              |

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Growth hormone family

|   |                          |   |     |   |   |       |  |
|---|--------------------------|---|-----|---|---|-------|--|
|   | GH                       | ? | -   | + | - | 5     |  |
|   | PRL                      | ? | +/- | + | - | 1,3,5 |  |
| 5 | EPO                      | ? | -   | + | - | 5     |  |
|   | GAS(B-CAS>IRF1=IFP>>Ly6) |   |     |   |   |       |  |

Receptor Tyrosine Kinases

|    |       |   |   |   |   |     |                |
|----|-------|---|---|---|---|-----|----------------|
|    | EGF   | ? | + | + | - | 1,3 | GAS (IRF1)     |
| 10 | PDGF  | ? | + | + | - | 1,3 |                |
|    | CSF-1 | ? | + | + | - | 1,3 | GAS (not IRF1) |

15 To construct a synthetic GAS containing promoter element, which is used in the biological assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS-binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman, *et al.*, *Immunity* 1:457-468 (1994)), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18 bp of sequence complementary to the SV40 early promoter sequence and is flanked with an *Xho* I restriction site. The sequence of the 5' primer is: 5'-GCG CCT CGA GAT TTC CCC GAA ATC TAG ATT TCC CCG AAA TGA TTT CCC CGA AAT GAT TTC CCC GAA ATA TCT GCC ATC TCA ATT AG-3' (SEQ ID NO:15).

25 The downstream primer is complementary to the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA GGC-3' (SEQ ID NO:16).

30 PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene). Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

35 CTCGAGATTTCCCCGAAATCTAGATTTC~~CCCGAAATGATTTC~~CCCGAAATATCTGCCATCTCAATTAGT  
CAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCCGCCATTCTCCGCCCATGGCTGA  
CTAATTTTATTTATTTATGAGAGGCGAGGCGGCTCGGCTCTGAGCTATTCCAGAAGT/AGTGAGGAGGCTTTTGGAGG  
CCTAGGCTTTTGCAAAAGCTT (SEQ ID NO:17).

40 With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP". Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase,

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alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using *Hin* dIII and *Xho* I, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using *Sal* I and *Not* I, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NF- $\kappa$ B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HeLa (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

25

#### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity of IL-21 by determining whether IL-21 supernatant proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies; transfection procedure described below). The transfected cells are seeded to a density of approximately

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20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells

5 containing 200  $\mu$ l of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10  $\mu$ g of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50  $\mu$ l of DMRIE-C and incubate at room temperature for 15-45 min.

10 During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37°C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

15 The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing IL-21 polypeptides or IL-21 induced polypeptides as produced by the protocol described in Example 11.

20 On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

25 Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200  $\mu$ l of cells into each well (therefore adding 100,000 cells per well).

30 After all the plates have been seeded, 50  $\mu$ l of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

35 The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35  $\mu$ l samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane covers) and stored at -20°C until SEAP assays are performed according to Example 17. The plates

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containing the remaining treated cells are placed at 4°C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity of IL-21 by determining whether IL-21 proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda, *et. al.*, *Cell Growth & Differentiation*, 5:259-265 (1994)) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 µg GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 µM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 µM  $\text{CaCl}_2$ . Incubate at 37°C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37°C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 µg/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 µg/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 µl above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 µl cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add 50 µl of the supernatant prepared by the protocol described in Example 11. Incubate at 37°C for 48 to 72 hr. As a positive control, 100 U/ml interferon gamma can be used which is known to activate U937 cells. Over 30-fold induction is typically

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observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

**Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by IL-21.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by IL-21 can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (nucleotides -633 to +1; Sakamoto, K., *et al.*, *Oncogene* 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers: (A) 5' Primer: 5'-GCG CTC GAG GGA TGA CAG CGA TAG AAC CCC GG-3' (SEQ ID NO:18) and (B) 3' Primer: 5'-GCG AAG CTT CGC GAC TCC CCG GAT CCG CCT C-3' (SEQ ID NO:19).

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes *Xho* I and *Hin* dIII, removing the GAS/SV40 stuffer fragment. Digest the EGR1 amplified product with the same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, 2 ml of a coating solution (1:30 dilution of collagen type-I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin on a precoated 10 cm tissue culture dish. A 1:4 split is

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done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by  
 5 growing the cells in 300  $\mu\text{g/ml}$  G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300  $\mu\text{g/ml}$  G418 for several passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS.  
 10 Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$   
 15 cells/ml.

Add 200  $\mu\text{l}$  of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50  $\mu\text{l}$  supernatant produced by Example 11, 37° C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ $\mu\text{l}$  of Neuronal Growth Factor (NGF). Over fifty-fold  
 20 induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF- $\kappa\text{B}$  (Nuclear Factor  $\kappa\text{B}$ ) is a transcription factor activated by a wide variety  
 25 of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin- $\alpha$  and lymphotoxin- $\beta$ , by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF- $\kappa\text{B}$  regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- $\kappa\text{B}$  appears to shield cells from apoptosis), B- and T-cell development, anti-viral and  
 30 antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-  $\kappa\text{B}$  is retained in the cytoplasm with I- $\kappa\text{B}$  (Inhibitor  $\kappa\text{B}$ ). However, upon stimulation, I-  $\kappa\text{B}$  is phosphorylated and degraded, causing NF-  $\kappa\text{B}$  to shuttle to the nucleus, thereby activating transcription of target  
 35 genes. Target genes activated by NF-  $\kappa\text{B}$  include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

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Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF- $\kappa$ B promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF- $\kappa$ B would be useful in treating diseases. For example, inhibitors of NF- $\kappa$ B could be used to treat those diseases

5 related to the acute or chronic activation of NF- $\kappa$ B, such as rheumatoid arthritis.

To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (5'-GGG GAC TTT CCC-3'; SEQ ID NO:20), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with

10 an *Xho* I site: 5'-GCG GCC TCG AGG GGA CTT TCC CGG GGA CTT TCC GGG GAC TTT CCG GGA CTT TCC ATC CTG CCA TCT CAA TTA G-3' (SEQ ID NO:21).

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA

15 GGC-3' (SEQ ID NO:22).

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene).

Sequencing with the T7 and T3 primers confirms the insert contains the following

20 sequence:

5'-CTCGAGGGGACTTTCCCGGGGACTTTCCGGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAG  
TCCGCCCCCTAACTCCGCCATCCGCCCTAACTCCGCCAGTTCCGCCATTTCCGCCCATGGCTGACTAATTTT  
ATTATGCAGAGGCCGAGGCCGCTCGGCTCTGAGCTATTCCAGAGTAGTGAGGAGGCTTTTTCGAGGCCCTAGGCTTTTG  
25 CAAAAAGCTT-3' (SEQ ID NO:23)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF- $\kappa$ B/SV40 fragment using *Xho* I and *Hin* dIII. However, this vector does not contain a neomycin resistance gene, and

30 therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF- $\kappa$ B/SV40/SEAP cassette is removed from the above NF- $\kappa$ B/SEAP vector using restriction enzymes *Sal* I and *Not* I, and inserted into a vector containing neomycin resistance. Particularly, the NF- $\kappa$ B/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP

35 gene, after restricting pGFP-1 with *Sal* I and *Not* I.

Once NF- $\kappa$ B/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the

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method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF- $\alpha$  (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### 5 **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

- 10 Prime a dispenser with the 2.5x Dilution Buffer and dispense 15  $\mu$ l of 2.5x dilution buffer into Optiplates containing 35  $\mu$ l of a supernatant. Seal the plates with a plastic sealer and incubate at 65°C for 30 min. Separate the Optiplates to avoid uneven heating.

- 15 Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50  $\mu$ l Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50  $\mu$ l Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each
- 20 time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

| # of plates | Rxn buffer diluent (ml) | CSPD (ml) |
|-------------|-------------------------|-----------|
| 10          | 60                      | 3         |
| 11          | 65                      | 3.25      |
| 12          | 70                      | 3.5       |
| 13          | 75                      | 3.75      |
| 14          | 80                      | 4         |
| 15          | 85                      | 4.25      |
| 16          | 90                      | 4.5       |
| 17          | 95                      | 4.75      |
| 18          | 100                     | 5         |
| 19          | 105                     | 5.25      |
| 20          | 110                     | 5.5       |
| 21          | 115                     | 5.75      |
| 22          | 120                     | 6         |
| 23          | 125                     | 6.25      |
| 24          | 130                     | 6.5       |
| 25          | 135                     | 6.75      |
| 26          | 140                     | 7         |
| 27          | 145                     | 7.25      |

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|    |     |       |
|----|-----|-------|
| 28 | 150 | 7.5   |
| 29 | 155 | 7.75  |
| 30 | 160 | 8     |
| 31 | 165 | 8.25  |
| 32 | 170 | 8.5   |
| 33 | 175 | 8.75  |
| 34 | 180 | 9     |
| 35 | 185 | 9.25  |
| 36 | 190 | 9.5   |
| 37 | 195 | 9.75  |
| 38 | 200 | 10    |
| 39 | 205 | 10.25 |
| 40 | 210 | 10.5  |
| 41 | 215 | 10.75 |
| 42 | 220 | 11    |
| 43 | 225 | 11.25 |
| 44 | 230 | 11.5  |
| 45 | 235 | 11.75 |
| 46 | 240 | 12    |
| 47 | 245 | 12.25 |
| 48 | 250 | 12.5  |
| 49 | 255 | 12.75 |
| 50 | 260 | 13    |

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

For adherent cells, seed the cells at 10,000-20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 µl of HBSS (Hank's Balanced Salt Solution) leaving 100 µl of buffer after the final wash.

A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 µl of 12 µg/ml fluo-3 is added to each well. The plate is

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incubated at 37°C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 µl of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. Four µl of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each 1 ml of cell suspension. The tube is then placed in a 37°C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 µl/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 µl, followed by an aspiration step to 100 µl final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 µl. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either IL-21 or a molecule induced by IL-21, which has resulted in an increase in the intracellular Ca<sup>2+</sup> concentration.

#### 20 **Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., *src*, *yes*, *lck*, *lyn*, *fyn*) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

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Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether IL-21 or a molecule induced by IL-21 is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4°C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamar Blue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes, treatment with EGF (60ng/ml) or 50 µl of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4°C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include

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PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

- The tyrosine kinase reaction is set up by adding the following components in order. First, add 10  $\mu$ l of 5  $\mu$ M Biotinylated Peptide, then 10  $\mu$ l ATP/Mg<sup>2+</sup> (5 mM ATP/50 mM MgCl<sub>2</sub>), then 10  $\mu$ l of 5x Assay Buffer (40 mM imidazole hydrochloride, pH 7.3, 40 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 100 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5  $\mu$ l of Sodium Vanadate (1 mM), and then 5  $\mu$ l of water. Mix the components gently and preincubate the reaction mix at 30°C for 2 min. Initial the reaction by adding 10  $\mu$ l of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10  $\mu$ l of 120 mM EDTA and place the reactions on ice.

- Tyrosine kinase activity is determined by transferring 50  $\mu$ l aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37°C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300  $\mu$ l/well of PBS four times. Next add 75  $\mu$ l of anti-phosphotyrosine antibody conjugated to horse radish peroxidase (anti-P-Tyr-POD (0.5  $\mu$ l/ml)) to each well and incubate at 37°C for one hour. Wash the well as above.

- Next add 100  $\mu$ l of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 min (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### Example 20: High-Throughput Screening Assay Identifying

##### 25 Phosphorylation Activity

- As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

- Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1 ml of protein G (1  $\mu$ g/ml) for 2 hr at room temp (RT). The plates are

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then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100 ng/well) against Erk-1 and Erk-2 (1 hr at RT; available from Santa Cruz Biotechnology). To detect other molecules, this step can easily be modified by substituting a monoclonal antibody  
 5 detecting any of the above described molecules. After 3-5 rinses with PBS, the plates are stored at 4°C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6 ng/well) or 50 µl of the supernatants  
 10 obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10 ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody  
 15 (1 µg/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over  
 20 background indicates a phosphorylation by IL-21 or a molecule induced by IL-21.

#### **Example 21: Method of Determining Alterations in the IL-21 Gene**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from  
 25 these RNA samples using protocols known in the art (see, Sambrook, *et al.*, *supra*). The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described by Sidransky and colleagues (*Science* 252:706 (1991)).

30 PCR products are then sequenced using primers labeled at the 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon borders of selected exons of IL-21 are also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in IL-21 are then cloned and sequenced to validate the results of the direct  
 35 sequencing.

PCR products of IL-21 are cloned into T-tailed vectors as described by Holton and Graham (*Nucl. Acids Res.* 19:1156 (1991)) and sequenced with T7 polymerase

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(United States Biochemical). Affected individuals are identified by mutations in IL-21 not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in the IL-21 gene. Genomic clones isolated according to Example 2 are  
 5 nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim),  
 and FISH performed as described by Johnson and coworkers (*Methods Cell Biol.*  
 35:73-99 (1991)). Hybridization with the labeled probe is carried out using a vast  
 excess of human cot-1 DNA for specific hybridization to the IL-21 genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and  
 10 propidium iodide, producing a combination of C- and R-bands. Aligned images for  
 precise mapping are obtained using a triple-band filter set (Chroma Technology,  
 Brattleboro, VT) in combination with a cooled charge-coupled device camera  
 (Photometrics, Tucson, AZ) and variable excitation wavelength filters (Johnson, C., *et*  
*al.*, *Genet. Anal. Tech. Appl.* 8:75 (1991)). Image collection, analysis and  
 15 chromosomal fractional length measurements are performed using the ISee Graphical  
 Program System. (Inovision Corporation, Durham, NC). Chromosome alterations of  
 the genomic region of IL-21 (hybridized by the probe) are identified as insertions,  
 deletions, and translocations. These IL-21 alterations are used as a diagnostic marker  
 for an associated disease.

#### Example 22: Method of Detecting Abnormal Levels of IL-21 in a Biological Sample

IL-21 polypeptides can be detected in a biological sample, and if an increased or  
 decreased level of IL-21 is detected, this polypeptide is a marker for a particular  
 25 phenotype. Methods of detection are numerous, and thus, it is understood that one  
 skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect IL-21 in a sample,  
 preferably a biological sample. Wells of a microtiter plate are coated with specific  
 antibodies to IL-21, at a final concentration of 0.2 to 10 µg/ml. The antibodies are  
 30 either monoclonal or polyclonal and are produced by the method described in Example  
 10. The wells are blocked so that non-specific binding of IL-21 to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample  
 containing IL-21. Preferably, serial dilutions of the sample should be used to validate  
 results. The plates are then washed three times with deionized or distilled water to  
 35 remove unbound IL-21.

Next, 50 µl of specific antibody-alkaline phosphatase conjugate, at a  
 concentration of 25-400 ng, is added and incubated for 2 hours at room temperature.

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The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

Add 75  $\mu$ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot IL-21 polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the IL-21 in the sample using the standard curve.

#### **Example 23: Formulating a Polypeptide**

The IL-21 composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the IL-21 polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of IL-21 administered parenterally per dose will be in the range of about 1  $\mu$ g/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, IL-21 is typically administered at a dose rate of about 1  $\mu$ g/kg/hour to about 50  $\mu$ g/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing IL-21 are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

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IL-21 is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U., *et al.*, *Biopolymers* 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer, R., *et al.*, *J. Biomed. Mater. Res.* 15:167-277 (1981); Langer, R. *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer, R., *et al.*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped IL-21 polypeptides. Liposomes containing the IL-21 are prepared by methods known *per se* (DE 3,218,121; Epstein, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang, *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, IL-21 is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting IL-21 uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or

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immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

IL-21 is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

IL-21 used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

IL-21 polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous IL-21 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized IL-21 polypeptide using bacteriostatic Water-For-Injection (WFI).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, IL-21 may be employed in conjunction with other therapeutic compounds.

#### **Example 24: Method of Treating Decreased Levels of IL-21**

The present invention relates to a method for treating an individual in need of a decreased level of IL-21 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of IL-21 antagonist. Preferred antagonists for use in the present invention are IL-21-specific antibodies.

Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of IL-21 in an individual can be treated by

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administering IL-21, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of IL-21 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of IL-21 to increase the activity level of IL-21 in such an individual.

For example, a patient with decreased levels of IL-21 polypeptide receives a daily dose 0.1-100 µg/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

#### **Example 25: Method of Treating Increased Levels of IL-21**

The present invention also relates to a method for treating an individual in need of an increased level of IL-21 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of IL-21 or an agonist thereof.

Antisense technology is used to inhibit production of IL-21. This technology is one example of a method of decreasing levels of IL-21 polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of IL-21 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### **Example 26: Method of Treatment Using Gene Therapy**

One method of gene therapy transplants fibroblasts, which are capable of expressing IL-21 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T., *et al.*, *DNA* 7:219-25 (1988)), flanked by the long  
5 terminal repeats of the Moloney murine sarcoma virus, is digested with *Eco* RI and *Hin*  
dIII and subsequently treated with calf intestinal phosphatase. The linear vector is  
fractionated on agarose gel and purified, using glass beads.

The cDNA encoding IL-21 can be amplified using PCR primers which  
correspond to the 5' and 3' end sequences respectively as set forth in Example 1.  
10 Preferably, the 5' primer contains an *Eco* RI site and the 3' primer includes a *Hin* dIII  
site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the  
amplified *Eco* RI and *Hin* dIII fragment are added together, in the presence of T4 DNA  
ligase. The resulting mixture is maintained under conditions appropriate for ligation of  
the two fragments. The ligation mixture is then used to transform bacteria HB101,  
15 which are then plated onto agar containing kanamycin for the purpose of confirming  
that the vector contains properly inserted IL-21.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue  
culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10%  
calf serum (CS), penicillin and streptomycin. The MSV vector containing the IL-21  
20 gene is then added to the media and the packaging cells transduced with the vector. The  
packaging cells now produce infectious viral particles containing the IL-21 gene (the  
packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the  
media is harvested from a 10 cm plate of confluent producer cells. The spent media,  
25 containing the infectious viral particles, is filtered through a millipore filter to remove  
detached producer cells and this media is then used to infect fibroblast cells. Media is  
removed from a sub-confluent plate of fibroblasts and quickly replaced with the media  
from the producer cells. This media is removed and replaced with fresh media. If the  
titer of virus is high, then virtually all fibroblasts will be infected and no selection is  
30 required. If the titer is very low, then it is necessary to use a retroviral vector that has a  
selectable marker, such as neo or his. Once the fibroblasts have been efficiently  
infected, the fibroblasts are analyzed to determine whether IL-21 protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or  
after having been grown to confluence on cytodex 3 microcarrier beads.

35 It will be clear that the invention may be practiced otherwise than as particularly  
described in the foregoing description and examples. Numerous modifications and

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variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other  
5 disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

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### Sequence Listing

(1) GENERAL INFORMATION:

- (i) APPLICANT: Ebner, Reinhard  
Ruben, Steven M.
- (ii) TITLE OF INVENTION: Interleukins 21 and 22
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Human Genome Sciences, Inc.
  - (B) STREET: 9410 Key West Avenue
  - (C) CITY: Rockville
  - (D) STATE: MD
  - (E) COUNTRY: US
  - (F) ZIP: 20850
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Brookes, A. Anders
  - (B) REGISTRATION NUMBER: 36,373
  - (C) REFERENCE/DOCKET NUMBER: PF470PP
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 301-309-8504
  - (B) TELEFAX: 301-309-8439

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 703 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCAGGATG GACACGATG AGGACCGCTA TCCACAGAAG CTGGCCCTTCG CCGAGTACT

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GTGCAGAGGC TGTATCGATG CACGGACGGG CCGGAGACA GCTGGCTCA ACTCCGTGG 120  
 GCTGCTCCAG AGCCTGCTGG TGCTGGCCG CCGGCTGTC TCCCGGACG GCTCGGGGT 180  
 CCCCACACCT GGGGCTTTG CCTTCCACAC CGAGTTCATC CAGTCCCCG TGGGCTGCAC 240  
 CTGCGTGTG CCCCCTTCAG TGTACCCGC AAGGCCGTGG GGCCCTTAGA CTGGACAGT 300  
 GTGCTCCCCA GAGGGCACCC CCTATTTATG TGTATTTATT GTTATTTATA TGCCTCCCCC 360  
 AACACTACCC TTGGGGTCTG GGCATTCCCC GTGTCTGGAG GACAGCCCCC CACTGTTCTC 420  
 CTCATCTCCA GCCTCAGTAG TTGGGGTGA AGGAGCTCAG CACCTCTTCC AGCCCTTAAA 480  
 GCTGCAGAAA AGGTGTACA CGGCTGCTG TACCTTGGTC CCGTCTCTG TCCCGGCTC 540  
 CCTTACCCTA TCACTGGCCT CAGGCCCCG CAGGCTGCCT CTTCCTCAAC TCCTTGAAG 600  
 TACCCCTGTT TCTTAAACAA TTATTTAAGT GTACGTGTAT TATTAACTG ATGAACACAA 660  
 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA 703

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 87 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Arg Val Asp Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu Ala Phe  
 1 5 10 15  
 Ala Glu Cys Leu Cys Arg Gly Cys Ile Asp Ala Arg Thr Gly Arg Glu  
 20 25 30  
 Thr Ala Ala Leu Asn Ser Val Arg Leu Leu Gln Ser Leu Leu Val Leu  
 35 40 45  
 Arg Arg Arg Pro Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr Pro Gly  
 50 55 60  
 Ala Phe Ala Phe His Thr Glu Phe Ile His Val Pro Val Gly Cys Thr  
 65 70 75 80  
 Cys Val Leu Pro Arg Ser Val  
 85

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1642 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

|  |      |
|--|------|
| GGAATTCGGC ACGAGCTCGT GCCGTGCTCA GTGCCTTCCA CCACACGCIG CAGCTGGGGC  | 60   |
| CGCGTGAGCA GCGCGCAAC GCGAGCTGCC CGGCAGGGGG CAGGCCCGCC GACCGCCGCT   | 120  |
| TCCGGCGGCC CACCAACCTG CGCAGCGTGT CGCCCTGGGC CTACAGAATC TCCTACGACC  | 180  |
| CGCGAGGTA CCCAGGTAC CTGCCTGAAG CCTACTGCCT GTCCCGGGGC TGCTTGACCG    | 240  |
| GGCTGTTCGG CGAGGAGGAC GTGCGCTTCC GCAGCGCCCC TGTCTACATG CCCACCGTCG  | 300  |
| TCCTGCGCGC CACCCCGGCC TGCGCCGGCG GCGTTCGGT CTACACCGAG GCCTACGTCA   | 360  |
| CCATCCCGGT GGGCTGCACC TGGTCCCGG AGCCGAGAGG GGACGCAGAC AGCATCAACT   | 420  |
| CCAGCATCGA CAAACAGGGC GCCAAGCTCC TGCTGGGGCC CAACGACGCG CCGCTGGGCC  | 480  |
| CCTGAGGCGG GTCTGCCCC GGGAGGTCTC CCGGCGCCGC ATCCCGAGGC GCUCAAGCTG   | 540  |
| GAGCGCGCTG GAGGCTCGG TCGGCGACCT CTGAAGAGAG TGACCGAGC AAACCAAGTG    | 600  |
| CGGAGACACC AGCGCCGCTT TCCTATGGAG ACTCGTAAGC AGCTTCATCT GACACGGGCA  | 660  |
| TCCTGGCTT GCTTTAGCT ACAAGCAAGC AGCGTGGCTG GAAGCTGATG GGAAACGACC    | 720  |
| CGGCACGGGC ATCTGTGTG CGGCCCGCAT GGAGGGTTTG GAAAAGTTCA CGGAGGCTCC   | 780  |
| CTGAGGAGCC TCTCAGATCG GCTGCTGGG GTCCAGGGCG TGACTCACCC CTGGGTGCTT   | 840  |
| GCCAAAGAGA TAGGGACGCA TATGCTTTT AAAGCAATCT AAAAATAATA ATAAGTATAG   | 900  |
| CGACTATATA CCTACTTTTA AAATCAACTG TTTTGAATAG AGGCAGAGCT ATTTATATTT  | 960  |
| ATCAAAATGAG AGCTACTCTG TTACATTTCT TAACATATAA ACATCGTTTT TTACTTCTTC | 1020 |
| TGGTAGAATT TTTTAAAGCA TAATTGGAAT CCTTGGATAA ATTTTGTAGC TGTACACTC   | 1080 |
| TGGCTGGGT CTCTGAATTC AGCCTGTCAC CGATGGCTGA CTGATGAAAT GGACACGTCT   | 1140 |
| CATCTGACCC ACTCTTCCTT CCACTGAAGG TCTTCACGG CCTCCAGGTG GACCAAAGGG   | 1200 |
| ATGCACAGGC GGCTCGCATG CCCCAGGGCC AGCTAAGACT TCCAAGATC TCAGATTTTG   | 1260 |
| TTTTAGTCAT GAATACATAA ACAGTCTCAA ACTCGCACAA TTTTTTCCCC CTTTTGAAAG  | 1320 |
| CCACTGGGC CAATTTGTGG TTAAGAGGTG GTGAGATAAG AAGTGAACG TGACATCTTT    | 1380 |
| GCCAGTGTG AGAAGAAATC AAGCAGGTAT TGGCTTAGTT GTAAGGCTT TAGGATCAGG    | 1440 |
| CTGAATATGA GGACAAAGTG GGCCAGTTA GCATCTGCAG AGATCAATCT GGAGGCTTCT   | 1500 |
| GTTCCTGCAT TCTGCCAGG GAGCTAGGTC CTGATCTTCT TCTTAGATT GAAAGTCGT     | 1560 |

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CTCTGAACAC AATTATTGT AAAAGTTAGT AGTTCTTTT TAAATCATT AAAGAGGCTT 1620

GCTGAAAAA AAAAAAAAAA AA 1642

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 160 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Ser | Ala | Arg | Ala | Arg | Ala | Val | Leu | Ser | Ala | Phe | His | His | Thr | Leu |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |
| Gln | Leu | Gly | Pro | Arg | Glu | Gln | Ala | Arg | Asn | Ala | Ser | Cys | Pro | Ala | Gly |
|     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |     |     |     |     |
| Gly | Arg | Pro | Ala | Asp | Arg | Arg | Phe | Arg | Pro | Pro | Thr | Asn | Leu | Arg | Ser |
|     | 35  |     |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Val | Ser | Pro | Trp | Ala | Tyr | Arg | Ile | Ser | Tyr | Asp | Pro | Ala | Arg | Tyr | Pro |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Arg | Tyr | Leu | Pro | Glu | Ala | Tyr | Cys | Leu | Cys | Arg | Gly | Cys | Leu | Thr | Gly |
| 65  |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |     |
| Leu | Phe | Gly | Glu | Glu | Asp | Val | Arg | Phe | Arg | Ser | Ala | Pro | Val | Tyr | Met |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     |     | 95  |     |
| Pro | Thr | Val | Val | Leu | Arg | Arg | Thr | Pro | Ala | Cys | Ala | Gly | Gly | Arg | Ser |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
| Val | Tyr | Thr | Glu | Ala | Tyr | Val | Thr | Ile | Pro | Val | Gly | Cys | Thr | Cys | Val |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Pro | Glu | Pro | Glu | Lys | Asp | Ala | Asp | Ser | Ile | Asn | Ser | Ser | Ile | Asp | Lys |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Gln | Gly | Ala | Lys | Leu | Leu | Leu | Gly | Pro | Asn | Asp | Ala | Pro | Ala | Gly | Pro |
| 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 155 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Thr Pro Gly Lys Thr Ser Leu Val Ser Leu Leu Leu Leu Ser  
 1 5 10 15

Leu Glu Ala Ile Val Lys Ala Gly Ile Thr Ile Pro Arg Asn Pro Gly  
 20 25 30

Cys Pro Asn Ser Glu Asp Lys Asn Phe Pro Arg Thr Val Met Val Asn  
 35 40 45

Leu Asn Ile His Asn Arg Asn Thr Asn Thr Asn Pro Lys Arg Ser Ser  
 50 55 60

Asp Tyr Tyr Asn Arg Ser Thr Ser Pro Trp Asn Leu His Arg Asn Glu  
 65 70 75 80

Asp Pro Glu Arg Tyr Pro Ser Val Ile Trp Glu Ala Lys Cys Arg His  
 85 90 95

Leu Gly Cys Ile Asn Ala Asp Gly Asn Val Asp Tyr His Met Asn Ser  
 100 105 110

Val Pro Ile Gln Gln Glu Ile Leu Val Leu Arg Arg Glu Pro Pro His  
 115 120 125

Cys Pro Asn Ser Phe Arg Leu Glu Lys Ile Leu Val Ser Val Gly Cys  
 130 135 140

Thr Cys Val Thr Pro Ile Val His His Val Ala  
 145 150 155

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 158 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser Pro Gly Arg Ala Ser Ser Val Ser Leu Met Leu Leu Leu  
 1 5 10 15

Leu Ser Leu Ala Ala Thr Val Lys Ala Ala Ala Ile Ile Pro Gln Ser  
 20 25 30

Ser Ala Cys Pro Asn Thr Glu Ala Lys Asp Phe Leu Gln Asn Val Lys  
 35 40 45

Val Asn Leu Lys Val Phe Asn Ser Leu Gly Ala Lys Val Ser Ser Arg  
 50 55 60

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Arg Pro Ser Asp Tyr Leu Asn Arg Ser Thr Ser Pro Trp Thr Leu His  
65 70 75 80

Arg Asn Glu Asp Pro Asp Arg Tyr Pro Ser Val Ile Trp Glu Ala Gln  
85 90 95

Cys Arg His Gln Arg Cys Val Asn Ala Glu Gly Lys Leu Asp His His  
100 105 110

Met Asn Ser Val Leu Ile Gln Gln Glu Ile Leu Val Leu Lys Arg Glu  
115 120 125

Pro Glu Ser Cys Pro Phe Thr Phe Arg Val Glu Lys Met Leu Val Gly  
130 135 140

Val Gly Cys Thr Cys Val Ala Ser Ile Val Arg Gln Ala Ala  
145 150 155

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 151 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Thr Phe Arg Met Thr Ser Leu Val Leu Leu Leu Leu Ser Ile  
1 5 10 15

Asp Cys Ile Val Lys Ser Glu Ile Thr Ser Ala Gln Thr Pro Arg Cys  
20 25 30

Leu Ala Ala Asn Asn Ser Phe Pro Arg Ser Val Met Val Thr Leu Ser  
35 40 45

Ile Arg Asn Trp Asn Thr Ser Ser Lys Arg Ala Ser Asp Tyr Tyr Asn  
50 55 60

Arg Ser Thr Ser Pro Trp Thr Leu His Arg Asn Glu Asp Gln Asp Arg  
65 70 75 80

Tyr Pro Ser Val Ile Trp Glu Ala Lys Cys Arg Tyr Leu Gly Cys Val  
85 90 95

Asn Ala Asp Gly Asn Val Asp Tyr His Met Asn Ser Val Pro Ile Gln  
100 105 110

Gln Glu Ile Leu Val Val Arg Lys Gly His Gln Pro Cys Pro Asn Ser  
115 120 125

Phe Arg Leu Glu Lys Met Leu Val Thr Val Gly Cys Thr Cys Val Thr  
130 135 140

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Pro Ile Val His Asn Val Asp  
145 150

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 180 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Asp Trp Pro His Asn Leu Leu Phe Leu Leu Thr Ile Ser Ile Phe  
1 5 10 15  
 Leu Gly Leu Gly Gln Pro Arg Ser Pro Lys Ser Lys Arg Lys Gly Gln  
20 25 30  
 Gly Arg Pro Gly Pro Leu Ala Pro Gly Pro His Gln Val Pro Leu Asp  
35 40 45  
 Leu Val Ser Arg Met Lys Pro Tyr Ala Arg Met Glu Glu Tyr Glu Arg  
50 55 60  
 Asn Ile Glu Glu Met Val Ala Gln Leu Arg Asn Ser Ser Glu Leu Ala  
65 70 75 80  
 Gln Arg Lys Cys Glu Val Asn Leu Gln Leu Trp Met Ser Asn Lys Arg  
85 90 95  
 Ser Leu Ser Pro Trp Gly Tyr Ser Ile Asn His Asp Pro Ser Arg Ile  
100 105 110  
 Pro Val Asp Leu Pro Glu Ala Arg Cys Leu Cys Leu Gly Cys Val Asn  
115 120 125  
 Pro Phe Thr Met Gln Glu Asp Arg Ser Met Val Ser Val Pro Val Phe  
130 135 140  
 Ser Gln Val Pro Val Arg Arg Arg Leu Cys Pro Pro Pro Pro Arg Thr  
145 150 155 160  
 Gly Pro Cys Arg Gln Arg Ala Val Met Glu Thr Ile Ala Val Gly Cys  
165 170 175  
 Thr Cys Ile Phe  
180

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 45 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCGCGGAT CCGACACGGA TGAGGACCGC TATCCACAGA AGCTG

45

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCCAAGCTTT CACACTGAAC GGGGCAGCAC GCAGGTGCAG C

41

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGCCGCGGAT CGCCATCCG CACGAGTGA CACGG

35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGCGGTACCC ACTGAACGGG GCAGCACGC

29

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

866250.0+E78009

- (A) LENGTH: 733 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

|   |     |
|---|-----|
| GGGATCCGGA GCCCAAATCT TCTGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG | 60  |
| AATTGAGGG TGCACCGTCA GTCTTCCTCT TCCCCCAAA ACCCAAGGAC ACCCTCATGA   | 120 |
| TCTCCCGGAC TCTGAGGTC ACATGCGTGG TGGTGGACGT AAGCCACGAA GACCCTGAGG  | 180 |
| TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG | 240 |
| AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCTT CACCGTCTG CACCAGGACT  | 300 |
| GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA ACCCCCATCG | 360 |
| AGAAAACCAT CTCCAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC  | 420 |
| CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT | 480 |
| ATCCAAGCGA CATGCCCGTG GAGTGGGAGA GCAATGGGCA GCCGAGAAC AACTACAAGA  | 540 |
| CCACGCTCC CGTGCTGGAC TCCGACGGCT CCTTCTTCTT CTACAGCAAG CTCACCGTGG  | 600 |
| ACAAGAGCAG GTGGCAGCAG GGGAACTCTT TCTCATGCTC CGTGATGCAT GAGGCTCTGC | 660 |
| ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGAGTG CGACGGCCGC | 720 |
| GACTCTAGAG GAT  | 733 |

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

|                          |
|--------------------------|
| Trp Ser Xaa Trp Ser      |
| 1                      5 |

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 86 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCGCCTCGAG ATTTCOCOGA AATCTAGATT TCCCCGAAAT GATTTCOCOG AAATGATTTC 60  
 CCCGAAATAT CTGCCATCTC AATTAG 86

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCGGCAAGCT TTTTGCAAAG CTTAGGC 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 271 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCGAGATTT CCCCAGAAATC TAGATTTCCT CGAAATGATT TCCCCGAAAT GATTTCOCOG 60  
 AAATATCTGC CATCTCAATT AGTCAGCAAC CATAGTCCCG CCCTTAACCT CGCCCATCCC 120  
 GCCCCTAACT CCGCCCAATT CCGCCCATTC TCCGCCCAT GGCTGACTAA TTTTITTTAT 180  
 TTATGCAGAG GCCGAGGCCG CCTCGGCCTC TGAGCTATTC CAGAAGTAGT GAGGAGGCTT 240  
 TTTTGGAGGC CTAGGCTTTT GCAAAAAGCT T 271

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 32 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCGCTCGAGG GATGACAGCG ATAGAACCCC GG

32

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCGAAGCTTC GCGACTCCCC GGATCGCGCT C

31

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGGACTTTC CC

12

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGGCTCGA GGGGACTTTC CCGGGGACTT TCCGGGACT TTCCGGGACT TTCCATCCTG

60

CCATCTCAAT TAG

73

(2) INFORMATION FOR SEQ ID NO:22:

866250.04E/8008

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCGGCAAGCT TTTGCAAAG CCTAGGC

27

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 256 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTCGAGGGGA CTTCCCGGG GACTTCCGG GGACTTCCG GGACTTTCCA TCTGCCATCT 60  
 CAATTAGTCA GCAACCATAG TCCCGCCCT AACTCGCCC ATCCCGCCC TAACTCGCC 120  
 CAGTTCGGCC CATTCCTCCG CCCATGGCTG ACTAATTTT TTTATTTATG CAGAGGCCGA 180  
 GGCGCCTCG GCCTCTGAGC TATTCAGAA GTAGTGAGGA GCTTTTTTG GAGGCTAGG 240  
 CTTTTCGAAA AAGCTT 256

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 371 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

NAATTCGGCA NAGGNGAAA CGACCCGGCA NCGATNCCT GTGTCCGCC CGCATGGAGG 60  
 GTTTGGAAAA GTTCACGGNG GCTCCTGAG GACCTGGAG AATCGGGCTG CTGCGGGTCC 120  
 AAGGCGTGA CTCACCGCTG GGTGCTTGGC AAANAGGATA GGGACGCATA TGCTTTTAA 180  
 AGCAATCTAA AAATAATAAT AAGTATAGCG ACTATATACC TACTTTTAA ATCAACTGTT 240

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TTGAATAGAG GCAGAGCTTA TTTTATATTA TCCAAATGAG AGCTACTCTG TTNACATTIT 300  
 CTTTAAACAT TTAAACATN GNTTTTPTNA CTCTTNCCTG GGTNGGATIT TTTTAAAGG 360  
 CNTAATTGGG A 371

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 498 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AATTCGGCAG AGCCAGNCCG GAGAAGGACG CAGACAGCAT CAACTCCAGC ATCGACAAAC 60  
 AGGGCGCCAA GCTCCTGCTG GGCCCAACG ACGCGCCCGC TGGCCCCTGA AGGNCGGTTC 120  
 CTGCCCCGGG AGGTCTCCCC GGNCCCGCAT CCGAGGGCGC CCAAGCTGGA GCGGCTTGGA 180  
 GGNITCGGTC GCGACTCTG AAGAGAGTNC ACCGAGCAAA CCAAGTGCCG GAGCAACAGC 240  
 GNCNCITTT NCATGGAGAT TCGTAAGCAN TTTCATTTC ACANGGGAT CCCTGGTTTG 300  
 TTTTAGTGA CAAGCAAGCA NTGCGTGA AGTNGNTGGG GAAAGGANC CNAGGGATTC 360  
 TGTNTNGGG GCNTNTGGA CGCTTTTGA AAATTNAGG GGGTNCIGN GGTTTTPTA 420  
 ANATTGGNT TTTTAGGAT TNAAGGTTN NTTAACTGG GGTTTTTCN AANNGTGGG 480  
 GGATNTTTT TNAAGATT 498

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 178 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCTTCAGCAA GCTCTTTTA ATGATTAAA AAAGAACTTC TAACTTTAC AAATAATTGT 60  
 GTTCAGAGAC AGACTTTCAA TCTAAAGAAA AGATCAAGGN CCTAGCTCTN GTGGCAGAAT 120  
 GCAGAAACAG AAGCCNCCAG ATNGANCTCN GCAGATGCTA ACGNGGCCCA CTTTGTCC 178

## (2) INFORMATION FOR SEQ ID NO:27:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 264 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

|  |     |
|--|-----|
| GGCAGAGCCA AGCTCCTGCT GNGCCCCAA CGACGCGCC GCTGCCCCCT AAGGCCGGTT    | 60  |
| CCTGCCCCCG AAGGTCTCCC CGGCCCGCAT CCGAGGCGC CCAAGCTGGA GCCGCCTGGA   | 120 |
| GGGCTTCGGT NCGGCGAACC TCTGAAAGAG AAGTGCCACC GAGCAAACCA AGTGCCCGTA  | 180 |
| GCACCAGNCG CGCCTTTCCA TGGAGACTCG TAAGCAGCTT CATCTGANAC GGGAAATCCCT | 240 |
| GGTTTGCTTT TAGCTACAAG CAAG   | 264 |

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*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209666;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (g) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (h) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666 having biological activity;
- (i) a polynucleotide which is a variant of SEQ ID NO:1;
- (j) a polynucleotide which is an allelic variant of SEQ ID NO:1;
- (k) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:2; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(k), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:3 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209665;

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- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (g) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (h) a polynucleotide encoding a polypeptide of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665 having biological activity;
- (i) a polynucleotide which is a variant of SEQ ID NO:3;
- (j) a polynucleotide which is an allelic variant of SEQ ID NO:3;
- (k) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:4; and
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(k), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

4. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

5. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the coding sequence included in ATCC Deposit No: 209666.

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6. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:4 or the coding sequence included in ATCC Deposit No: 209665.

7. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in ATCC Deposit No: 209666.

8. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:3 or the cDNA sequence included in ATCC Deposit No: 209665.

9. The isolated nucleic acid molecule of claims 3 or 4, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

10. The isolated nucleic acid molecule of claims 5 or 6, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

11. A recombinant vector comprising the isolated nucleic acid molecule of claims 1 or 2.

12. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

13. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 2.

14. A recombinant host cell produced by the method of claim 12.

15. A recombinant host cell produced by the method of claim 13.

16. The recombinant host cell of claim 14 comprising vector sequences.

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17. The recombinant host cell of claim 15 comprising vector sequences.

18. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;
- (b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;
- (d) a polypeptide epitope of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;
- (e) a mature form of a secreted protein;
- (f) a full length secreted protein;
- (g) a variant of SEQ ID NO:2;
- (h) an allelic variant of SEQ ID NO:2; or
- (i) a species homologue of the SEQ ID NO:2.

19. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (b) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (d) a polypeptide epitope of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (e) a mature form of a secreted protein;
- (f) a full length secreted protein;
- (g) a variant of SEQ ID NO:4;
- (h) an allelic variant of SEQ ID NO:4; or
- (i) a species homologue of the SEQ ID NO:4.

20. The isolated polypeptide of claims 18 or 19, wherein the mature form or the full length secreted protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

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21. An isolated antibody that binds specifically to the isolated polypeptide of claims 18 or 19.

22. A recombinant host cell that expresses the isolated polypeptide of claim 18.

23. A recombinant host cell that expresses the isolated polypeptide of claim 19.

24. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 22 under conditions such that said polypeptide is expressed; and  
(b) recovering said polypeptide.

25. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 23 under conditions such that said polypeptide is expressed; and  
(b) recovering said polypeptide.

26. The polypeptide produced by claims 24 or 25.

27. A method for preventing, treating, or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of claims 18 or 19 or of the polynucleotide of claims 1 or 2.

28. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

- (a) determining the presence or absence of a mutation in the polynucleotide of claims 1 or 2;
- (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

29. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

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- (a) determining the presence or amount of expression of the polypeptide of claims 18 or 19 in a biological sample;
- (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

30. A method for identifying binding partner to the polypeptide of claims 18 or 19 comprising:

- (a) contacting the polypeptide of claims 18 or 19 with a binding partner; and
- (b) determining whether the binding partner effects an activity of the polypeptide.

31. The gene corresponding to the cDNA sequence of SEQ ID NO:2.

32. The gene corresponding to the cDNA sequence of SEQ ID NO:4.

33. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:1 in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

34. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:3 in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

35. The product produced by the method of claim 33.

36. The product produced by the method of claim 34.

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***Abstract***

The present invention relates to novel human proteins designated Interleukin-21 (IL-21) and Interleukin-22 (IL-22), and isolated polynucleotides encoding these proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human proteins.

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Figure 1  
Interleukin-21

```

1  GGCACGAGTGGACACGGATGAGGACCGCTATCCACAGAAGCTGGCCTTCGCCGAGTGCCCT 60
1  A R V D T D E D R Y P Q K L A F A E C L 20
      Domain I                               Domain II

61  GTGCAGAGGCTGTATCGATGCACGGACGGGCGGAGACAGCTGGCGTCAACTCCGTGCG 120
21  C E G C I D A R T G R E T A A L N S V R 40
      Domain II

121 GCTGCTCCAGAGCCTGCTGGTGCTGCGCCGCGGCGCTGCTCCCGCGACGGCTCGGGGCT 180
41  L L Q S L L V L R R P C S R D G S G L 60
      Domain III

181 CCCCACACCTGGGGCCTTGGCCTTCCACACCGAGTTCA1CCACGTCCCGTGGGTGCAC 240
61  P T P G A F A F H T E F I H V P V G C T 80
      Domain IV

241 CTGCGTGCTGCCCGTTCAAGTGACCGCCAAGGCGGTGGGGCCCTTAGACTGGACACGT 300
81  C V L P R S V 87
      Domain IV

301 GTGCTCCCAGAGGGCACCCCTATTTATGTGTATTTATGTTATTTATATGCTCCCCC 360

361 AACACTACCCCTGGGGTCTGGGCATTCCCGTGTCTGGAGGACAGCCCCCACTGTTCTC 420

421 CTCATCTCCAGCCTCAGTAGTGGGGGTGAAGGAGCTCAGCACCTCTTCCAGCCCTTAA 480

481 AGCTGCAGAAAAGGTGTCACACGGCTGCTGTACCTTGGYTCCCTGTCTGCTCCCGGT 540

541 TCCCTTACCTATCAC1GGCCTCAGGCCCGCAGGCTGCCTCTTCCCAACCTCCTTGA 600

601 AGTACCCCTGTCTTCTTAAACAATTATTTAAGTGTACGTGTATTATTAACTGATGAACAC 660

661 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA . 705

```

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866250-04E/8009

Figure 2  
Interleukin-22

```

1  GGAATTCGGCAAGAGCTCGTGCCGTGCTCAGTGCCCTCCACCACAGCTGCAGCTGGGGC  60
1  N S A R A R A V I S A F H H T L Q L G P  20

#

61  CGCGTGAGCAGGCGCGCAACCGGACCTGCCCGGCAGGGGCGAGGCCCGCCGACCGCCGCT  120
21  R E Q A R N A S C P A G G R P A D R R F  40

121  TCCGGCCGCGCCACCAACCTGGCGCAGCGTGTCGCCCTGGGCCTACAGAACTCCTACGACC  180
41  R P P T N L R S V S P W A Y R I S Y D P  60
                                     Domain I

181  CGCGAGGTACCCAGGTACCTGCCTGAAGCTACTGCCCTGTGCCGGGCTGCTGACCG  240
61  A R Y P R Y L P E A Y C L C R G C L T G  80
      Domain I                               Domain II

241  GGCTGTTCGGCGAGGAGGACGTGCGCTTCCGCGAGCGCCCTGTCTACATGCCACCGTGG  300
81  L F G E E D V R F R S A P V Y M P T V V  100
                                     Domain III

301  TCCGCGCCGACCCCCCGCTGCGCGCGCGCGCTTCCGTCTACACCGAGGCTAGGTCA  360
101  L R R T P A C A G G R S V Y T E A Y V T  120
      Domain III

#

361  CCATCCCGTGGGCTGCACCTGCTTCCCGAGCCGAGAGGAGGACGAGACAGCATCAACT  420
121  I P V G C T C V P E P E K D A D S I N S  140
      Domain IV

421  CCAGCATCGACAAACAGGGCGCCAAGCTCCTGCTGGGCCCCAAGCAGCGCCCGCTGGCC  480
141  S I D K Q G A K L L L G P N D A P A G P  160

481  CCTGAGGCCGCTCTGCCCGGGAGGTCTCCCGGCCCGCATCCCGAGGCGCCCAAGCTG  540

541  GAGCCGCTCGAGGGCTCGGTCCGCGACCTCTGAAGAGAGTGCACCGAGCAAACCAAGTG  600

601  CCGGAGCACCAGCGCCGCTTTCATGGAGACTCGTAAGCAGCTTCATCTGACACGGGCA  660

661  TCCCTGGCTTGCTTTTAGCTACAAGCAAGCAGCGTGGCTGGAAGCTGATGGGAAACGACC  720

721  OGGCACGGGCATCCTGTGTGGGCGCGCATGGAGGGTTTGGAAAAGTTACCGGAGGCTCC  780

781  CTGAGGAGCCTCTCAGATCGGCTGCTGCGGGTGCAGGGCTGACTCACCGCTGGGTGCTT  840

841  GCCAAAGAGATAGGGACGCATATGCTTTTAAAGCAATCTAAAAATAATAAAGTATAG  900

```

Figure 2 (continued)  
Interleukin-22

901 CGACTATATACCTACTTTTAAATCAACTGTTTTGAATAGAGGCAGAGCTATTTATATT 960  
961 ATCAAATGAGAGCTACTCTGTTACATTTCTTAACATATAACATCGTTTTTACTTCTTC 1020  
1021 TGGTAGAATTTTTTAAAGCATAATTGGAACTCCTGGATAAAATTTGTAGCTGGTACACTC 1080  
1081 TGGCTGGGTCTCTGAATTCAGCCTGTACCGATGGCTGACTGATGAAATGGACACCTCT 1140  
1141 CATCTGACCCACTCTTCTTCCACTGAAGTCTTCACTGGGCTCCAGGTGACCAAAGGG 1200  
1201 ATGCACAGGCGGCTCGCATGCCCGAGGCCAGCTAAGAGTTCCAAAGATCTCAGATTTGG 1260  
1261 TTTTAGTCATGAATACATAAACAGTCTCAAACCTGCACAATTTTTCCCCCTTTTGAAG 1320  
1321 CCACTGGGGCCAATTTGTGGTTAAGAGGTGGTGAGATAAGAAGTGAACGTGACATCTTT 1380  
1381 GCCAGTTGTGAGAAGAATCCAAGCAGGTATTTGGCTTAGTTGTAAAGGCTTTAGGATCAGG 1440  
1441 CTGAATATGAGGACAAAGTGGGCCACGTTAGCATCTGCAGAGATCAATCTGGAGGCTTCT 1500  
1501 GTTTCGCAATCTGCCACGAGAGCTAGGTCCCTTGATCTTTCTTTAGATTGAAAGTCTGT 1560  
1561 CTCTGAACACAATTATTTGTAAAAGTTAGTAGTCTTTTAAATCATTAAGAGAGGCTT 1620  
1621 GCTGAAAAA/AAAAAAAAAAAA 1642

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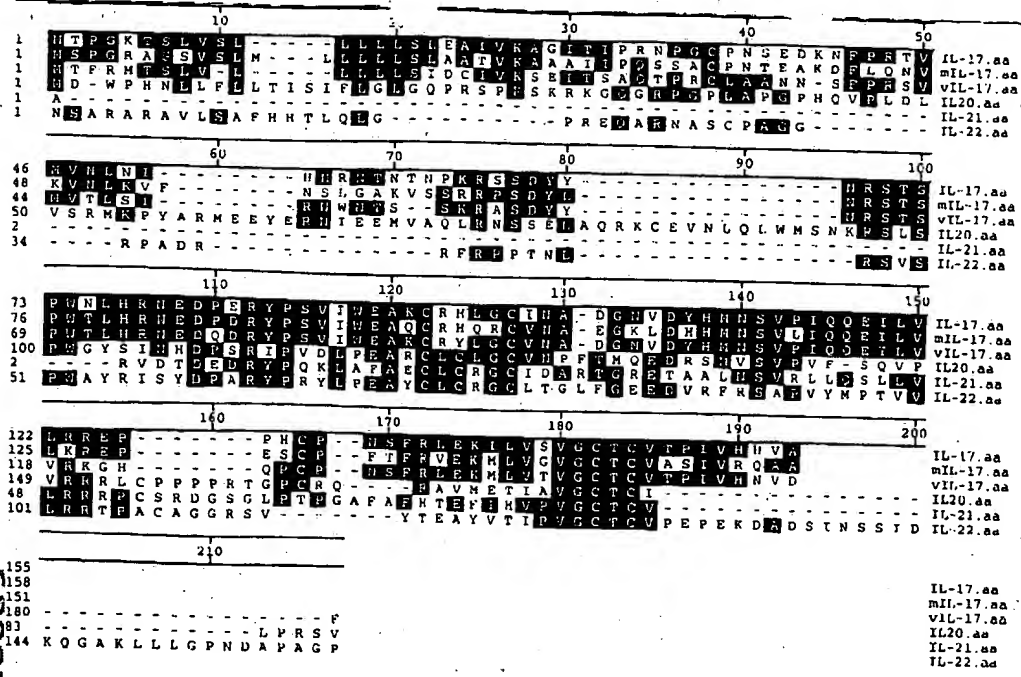
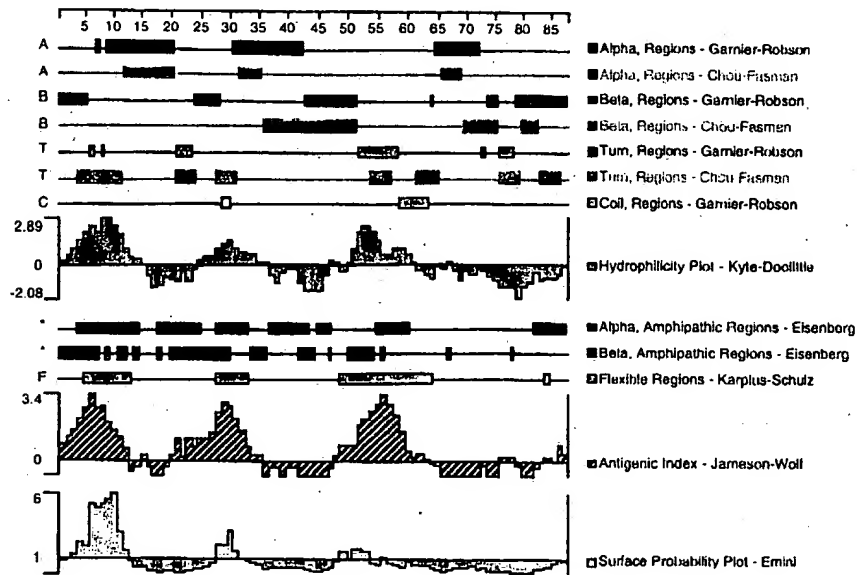


Figure 3

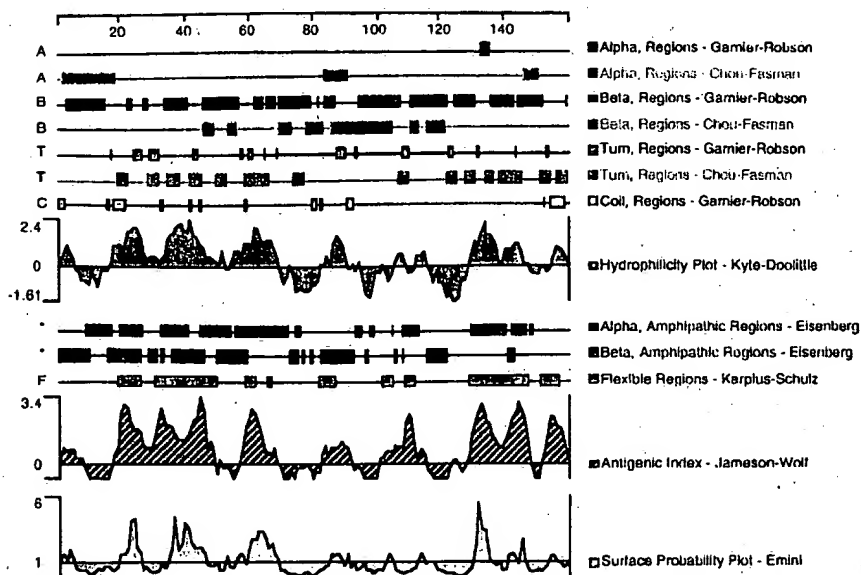


Figure 4  
Interleukin-21 Polypeptide Analysis



866250-04E/8009

Figure 5  
Interleukin-22 Polypeptide Analysis



866250-04E/8009

PTO/SS.22 (17-01)

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(19) **United States**(12) **Patent Application Publication**  
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## Publication Classification

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**C07H 21/04; C12N 15/09;**  
**C12P 21/02; C12P 21/04;**  
**A61K 45/00; A61K 39/395;**  
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(\*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: **09/320,713**(22) Filed: **May 27, 1999**(57) **ABSTRACT**

## Related U.S. Application Data

(60) Provisional application No. **60/087,340**, filed on **May 29, 1998**. Provisional application No. **60/099,805**, filed on **Sep. 10, 1998**. Provisional application No. **60/131,965**, filed on **Apr. 30, 1999**.

The present invention relates to novel human proteins designated Interleukin-21 (IL-21) and Interleukin-22 (IL-22), and isolated polynucleotides encoding these proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human proteins.



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| EBNER                          | Reinhard   |                | Gaithersburg, MD                                     |
| RUBEN                          | Steven     | M.             | Olney, MD  |
|                                |            |                |  |
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## Interleukins-21 and 22

### *Field of the Invention*

The present invention relates to two novel human genes, each of which encodes a polypeptide which is a member of the Interleukin family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Interleukin-21, or "IL-21". The present invention also relates to a polynucleotide encoding a novel human polypeptide named Interleukin-22, or "IL-22". This invention also relates to IL-21 and IL-22 polypeptides, as well as vectors, host cells, antibodies directed to IL-21 and IL-22 polypeptides, and recombinant methods for producing the same. Also provided are diagnostic methods for detecting disorders related to the immune system, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of IL-21 and IL-22 activity.

### *Background of the Invention*

Cytokines typically exert their respective biochemical and physiological effects by binding to specific receptor molecules. Receptor binding then stimulates specific signal transduction pathways (Kishimoto, T., *et al.*, *Cell* 76:253-262 (1994)). The specific interactions of cytokines with their receptors are often the primary regulators of a wide variety of cellular processes including activation, proliferation, and differentiation (Arai, K. -I, *et al.*, *Ann. Rev. Biochem.* 59:783-836 (1990); Paul, W. E. and Seder, R. A., *Cell* 76:241-251 (1994)).

Human interleukin (IL)-17, a closely related homolog of the molecules of the present invention, was only recently identified. IL-17 is a 155 amino acid polypeptide which was molecularly cloned from a CD4+ T-cell cDNA library (Yao, Z., *et al.*, *J. Immunol.* 155:5483-5486 (1995)). The IL-17 polypeptide contains an N-terminal signal peptide and contains approximately 72% identity at the amino acid level with a T-cell trophic herpesvirus saimiri (HVS) gene designated HVS13. High levels of IL-17 are secreted from CD4-positive primary peripheral blood leukocytes (PBL) upon stimulation (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Treatment of fibroblasts with IL-17, HVS13, or another murine homologue, designated CTLA8, activate signal transduction pathways and result in the stimulation of the NF- $\kappa$ B transcription factor family, the secretion of IL-6, and the costimulation of T-cell proliferation (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)).

An HVS13-Fc fusion protein was used to isolate a murine IL-17 receptor molecule which does not appear to belong to any of the previously described cytokine receptor families (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). The murine IL-17 receptor (mIL-17R) is predicted to encode a type I transmembrane protein of 864 amino acids with an apparent molecular mass of 97.8 kDa. mIL-17R is predicted to possess an N-terminal signal peptide with a cleavage site between alanine-31 and serine-32. The molecule also contains a 291 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 521 amino acid cytoplasmic tail. A soluble recombinant IL-17R molecule consisting of 323 amino acids of the extracellular domain of IL-17R fused to the Fc portion of human immunoglobulin IgG1 was able to significantly inhibit IL-17-induced IL-6 production by murine NIH-3T3 cells (*supra*).

Interestingly, the expression of the IL-17 gene is highly restricted. It is typically observed primarily in activated T-lymphocyte memory cells (Broxmeyer, H. J. *Exp. Med.* 183:2411-2415 (1996); Fossiez, F., *et al.*, *J. Exp. Med.* 183:2593-2603 (1996)). Conversely, the IL-17 receptor appears to be expressed in a large number of cells and tissues (Rouvier, E., *et al.*, *J. Immunol.* 150:5445-5456 (1993); Yao, Z., *et al.*, *J. Immunol.* 155:5483-5486 (1995)). It remains to be seen, however, if IL-17 itself can play an autocrine role in the expression of IL-17. IL-17 has been implicated as a causative agent in the expression of IL-6, IL-8, G-CSF, Prostaglandin E (PGE<sub>2</sub>), and intracellular adhesion molecule (ICAM)-1 (Fossiez, F., *supra*; Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Each of these molecules possesses highly relevant and potentially therapeutically valuable properties. For instance, IL-6 is involved in the regulation of hematopoietic stem and progenitor cell growth and expansion (Ikebuchi, K., *et al.*, *Proc. Natl. Acad. Sci. USA* 84:9035-9039 (1987); Gentile, P. and Broxmeyer, H. E. *Ann. N.Y. Acad. Sci. USA* 628:74-83 (1991)). IL-8 exhibits a myelosuppressive activity for stem cells and immature subsets of myeloid progenitors (Broxmeyer, H. E., *et al.*, *Ann. Hematol.* 71:235-246 (1995); Daly, T. J., *et al.*, *J. Biol. Chem.* 270:23282-23292 (1995)). G-CSF acts both early and late to activate and stimulate hematopoiesis in general, and more specifically on neutrophil hematopoiesis, while PGE<sub>2</sub> enhances erythropoiesis, suppresses lymphopoiesis and myelopoiesis in general, and strongly suppresses monocytopenia (Broxmeyer, H. E. *Amer. J. Ped. Hematol./Oncol.* 14:22-30 (1992); Broxmeyer, H. E. and Williams, D. E. *CRC Crit. Rev. Oncol./Hematol.* 8:173-226 (1988)).

Thus, there is a need for polypeptides that function as immunoregulatory molecules and, thereby, modulate the transfer of an extracellular signal ultimately to the nucleus of the cell, since disturbances of such regulation may be involved in disorders relating to cellular activation, hemostasis, angiogenesis, tumor metastasis, cellular

migration and ovulation, as well as neurogenesis. Therefore, there is a need for identification and characterization of such human polypeptides which can play a role in detecting, preventing, ameliorating or correcting such disorders.

### ***Summary of the Invention***

5       The present invention relates to novel polynucleotides and the encoded polypeptides of IL-21 and IL-22. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders related to the polypeptides, and therapeutic methods for treating such disorders. The invention  
10 further relates to screening methods for identifying binding partners of IL-21 and IL-22.

### ***Brief Description of the Drawings***

15       Figure 1 shows the partial nucleotide sequence (SEQ ID NO:1) and the deduced amino acid sequence (SEQ ID NO:2) of IL-21. The locations of conserved Domains I-IV (see below) are underlined and labeled as such.

20       Figures 2A and 2B show the nucleotide sequence (SEQ ID NO:3) and the deduced amino acid sequence (SEQ ID NO:4) of IL-22. The locations of conserved Domains I-IV (see below) are underlined and labeled as such. The locations of two potential N-linked glycosylation sites are identified by a bolded asparagine symbol (N) accompanied by a bolded pound sign (#) located above the initial nucleotide of the codon encoding the corresponding asparagine.

25       Figures 3A and 3B show the regions of identity between the amino acid sequence of a partial-length IL-21 protein, and the IL-22 protein (designated IL-21.aa and IL-22.aa in the figure), the full-length IL-21 protein (designated IL-21FL.aa in the figure) the amino acid sequence of IL-20 (designated IL20.aa in the figure and disclosed in copending U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ ID NO:8), and human Interleukin-17 (designated IL-17.aa in the figure; GenBank Accession No. U32659; SEQ ID NO:5), mouse Interleukin-17  
30 (designated mIL-17.aa in the figure; GenBank Accession No. U43088; SEQ ID NO:6), and viral Interleukin-17 (designated vIL-17.aa in the figure; GenBank Accession No. X64346; SEQ ID NO:7), as determined by the MegAlign component of the computer program DNA\*Star (DNASTAR, Inc.) using the default parameters.

35       Figure 4 shows an analysis of the partial IL-21 amino acid sequence (SEQ ID NO:2). Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity;

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amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the IL-21 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

Figure 5 shows an analysis of the IL-22 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the IL-22 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

The data presented in Figure 5 are also represented in tabular form in Table II. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIII. The column headings refer to the following features of the amino acid sequence presented in Figure 5 and Table II: "Res": amino acid residue of SEQ ID NO:4 or Figures 2A and 2B; "Position": position of the corresponding residue within SEQ ID NO:4 or Figures 2A and 2B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Alpha, Amphipathic Regions - Eisenberg; X: Beta, Amphipathic Regions - Eisenberg; XI: Flexible Regions - Karplus-Schulz; XII: Antigenic Index - Jameson-Wolf; and XIII: Surface Probability Plot - Emini.

Figures 6A and 6B show the nucleotide sequence (SEQ ID NO:28) and the deduced amino acid sequence (SEQ ID NO:29) of the full-length IL-21. The locations of conserved Domains I-IV (identical to those shown in Figure 1) and of conserved Domains V-VII are underlined and labeled as such. A predicted signal peptide from methionine-1 to alanine-18 is double underlined.

Figure 7 shows an analysis of a full-length IL-21 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index" or "Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of a full-length IL-21 protein, that is, regions from which epitope-bearing peptides of the invention can be determined. Polypeptides and

polynucleotides encoding polypeptides comprising the domains defined by these graphs are contemplated by the present invention.

The data presented in Figure 7 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 7 and Table I: "Res": amino acid residue of SEQ ID NO:29 or Figures 6A and 6B; "Position": position of the corresponding residue within SEQ ID NO:29 or Figures 6A and 6B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

### *Detailed Description*

#### Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. However, a nucleic acid contained in a clone that is a member of a library (e.g., a genomic or cDNA library) that has not been isolated from other members of the library (e.g., in the form of a homogeneous solution containing the clone and other members of the library) or which is contained on a chromosome preparation (e.g., a chromosome spread), is not "isolated" for the purposes of this invention.

In the present invention, a "secreted" IL-21 or IL-22 protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as an IL-21 or IL-22 protein released into the extracellular space without necessarily containing a signal sequence. If the IL-21 or IL-22 secreted protein is released into the extracellular space, the IL-21 or IL-22 secreted protein can undergo extracellular processing to produce a "mature" IL-21 or

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IL-22 protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, an IL-21 or IL-22 "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or in SEQ ID NO:3, respectively, or the cDNA contained within the respective clones deposited with the ATCC. For example, the IL-21 or IL-22 polynucleotide can contain the nucleotide sequence of the full-length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, an IL-21 or IL-22 "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

A representative clone containing all or most of the sequence for SEQ ID NO:1 (designated HTGED19) was deposited with the American Type Culture Collection ("ATCC") on March 5, 1998, and was given the ATCC Deposit Number 209666. In addition, a representative clone containing all or most of the sequence for SEQ ID NO:3 (designated HFPBX96) was also deposited with the ATCC on March 5, 1998, and was given the ATCC Deposit Number 209665. The ATCC is located at 10801 University Blvd., Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

An IL-21 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the cDNA within the deposited clone. Further, An IL-22 "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:3, the complement thereof, or the cDNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the IL-21 and the IL-22 polynucleotides at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE



(20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100  $\mu\text{g/ml}$  salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a polyA+ stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The IL-21 and IL-22 polynucleotides can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, the IL-21 and IL-22 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the IL-21 polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. IL-21 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

IL-21 and IL-22 polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The IL-21 and IL-22 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art.

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Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the IL-21 and IL-22 polypeptides, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given IL-21 or IL-22 polypeptide. Also, a given IL-21 or IL-22 polypeptide may contain many types of modifications. IL-21 or IL-22 polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic IL-21 and IL-22 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Scifter, *et al.*, *Meth. Enzymol.* 182:626-646 (1990); Rattan, *et al.*, *Ann. NY Acad. Sci.* 663:48-62 (1992)).

"SEQ ID NO:1" refers to an IL-21 polynucleotide sequence while "SEQ ID NO:2" refers to an IL-21 polypeptide sequence. Likewise, "SEQ ID NO:3" refers to an IL-22 polynucleotide sequence while "SEQ ID NO:4" refers to an IL-22 polypeptide sequence.

An IL-21 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-21 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In addition, an IL-22 polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of an IL-22 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose-dependency. In the case where dose-dependency does exist, it need not be identical to that of the IL-21 or IL-22



polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the IL-21 or IL-22 polypeptides (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the IL-21 polypeptide).

#### IL-21 and IL-22 Polynucleotides and Polypeptides

Clone HTGED19, encoding IL-21, was isolated from a cDNA library derived from apoptotic T-cells. This clone contains the entire coding region identified as SEQ ID NO:2. The deposited clone contains a cDNA having a total of 705 nucleotides, which encodes a partial predicted open reading frame of 87 amino acid residues (see Figure 1). The partial open reading frame begins at a point in the complete IL-21 ORF such that the "G" in position 1 of SEQ ID NO:1 is actually in position 3 of a coding triplet. As such, the partial predicted IL-21 polypeptide sequence is shown beginning in-frame with an alanine residue at position 1 of SEQ ID NO:2. The alanine residue at position 1 of SEQ ID NO:2 is encoded by nucleotides 2-4 of the nucleotide sequence shown as SEQ ID NO:1. The ORF shown as SEQ ID NO:2 ends at a stop codon at nucleotide position 263-265 of the nucleotide sequence shown as SEQ ID NO:1. The predicted molecular weight of the partial IL-21 protein should be about 9,558 Daltons.

An initial BLAST analysis of the expression of the IL-21 cDNA sequence against the HGS EST database has also revealed a highly specific expression of this cDNA clone. In such an analysis, the HTGED19 cDNA sequence appears to be found only in apoptotic T-cells. Thus, IL-21 appears to be expressed in a highly restricted pattern limited to apoptotic T-cells, and, for example, other subpopulations of lymphocytes or other cells in a state of activation or quiescence.

Clone HTGED19, encoding IL-21, was used to screen a panel of bacterial artificial chromosomes containing various segments of human genomic DNA (Research Genetics, Inc.). A positive clone was sequenced to identify potential splice donor and acceptor sites. Analysis of several sites revealed an upstream partial ORF that, when placed immediately 5' and in frame with the existing IL-21 DNA sequence, generated a complete ORF which encodes a polypeptide with additional sequence identity to the IL-17 family (See Figures 3A and 3B). A full-length IL-21 clone has been constructed from the HTGED19 cDNA clone and the upstream genetic material. The nucleotide sequence of the full-length IL-21 clone contains the entire coding region identified as SEQ ID NO:29. The resultant clone contains an insert having a total of 1067 nucleotides, which encodes a predicted open reading frame of 197 amino acid residues (see Figures 6A and 6B). The open reading frame begins at nucleotide position 34 in

the complete IL-21 polynucleotide shown as SEQ ID NO:28 (Figures 6A and 6B). The ORF ends at a stop codon at nucleotide position 625-627 of the nucleotide sequence shown as SEQ ID NO:28 (Figures 6A and 6B). The predicted molecular weight of the IL-21 polypeptide shown in Figures 6A and 6B and as SEQ ID NO:29 should be about 21,764 Daltons.

Further BLAST analysis of the expression of the full-length IL-21 cDNA sequence against the HGS EST database has also revealed a highly specific expression of this cDNA clone. In such an analysis, the full-length HTGED19 cDNA sequence appears to be found only in apoptotic T-cells. Thus, IL-21 appears to be expressed in a highly restricted pattern limited to apoptotic T-cells, and, for example, other subpopulations of lymphocytes or other cells in a state of activation or quiescence.

Clone HFPBX96, encoding IL-22, was isolated from a cDNA library derived from epileptic frontal cortex. This clone contains the entire coding region identified as SEQ ID NO:4. The deposited clone contains a cDNA having a total of 1,642 nucleotides, which encodes a partial predicted open reading frame of 160 amino acid residues (see Figure 2). The partial open reading frame begins at a point in the complete IL-22 ORF such that the "G" in position 1 of SEQ ID NO:3 is actually in position two of a coding triplet. As such, the partial predicted IL-22 polypeptide sequence is shown beginning in-frame with an asparagine residue at position 1 of SEQ ID NO:4. The asparagine residue at position 1 of SEQ ID NO:4 is encoded by nucleotides 3-5 of the nucleotide sequence shown as SEQ ID NO:3. The ORF shown as SEQ ID NO:4 ends at a stop codon at nucleotide position 483-485 of the nucleotide sequence shown as SEQ ID NO:3. The predicted molecular weight of the partial IL-22 protein should be about 17,436 Daltons.

Using BLAST and MegAlign analyses, SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:29 were each found to be highly homologous to several members of the Interleukin family. Particularly, SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:29 contain at least four domains homologous to the translation products of the human mRNA for Interleukin (IL)-20 (copending U.S. Provisional Application Serial No. 60/060,140; filed September 26, 1997; SEQ ID NO:8), IL-17 (GenBank Accession No. U32659; SEQ ID NO:5; see also Figures 3A and 3B), the murine mRNA for Interleukin (IL)-17 (GenBank Accession No. U43088; SEQ ID NO:6; see also Figures 3A and 3B), and the human viral mRNA for Interleukin (IL)-17 (GenBank Accession No. X64346; SEQ ID NO:7; see also Figures 3A and 3B). Specifically, the molecules of the present invention, in particular, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:29, share a high degree of sequence identity with IL-20, IL-17, mIL-17, and vIL-17 in the following conserved domains: (a) a predicted NXDPXRY domain (where X

represents any amino acid) located at about amino acids valine-3 to proline-11 of SEQ ID NO:2, serine-57 to proline-64 of SEQ ID NO:4, valine-113 to proline-121 of SEQ ID NO:29, and asparagine-79 to proline-86 of the human IL-17 amino acid sequence (SEQ ID NO:5); (b) a predicted CLCXGC domain (where X represents any amino acid) located at about amino acids cysteine-19 to cysteine-24 of SEQ ID NO:2, cysteine-72 to cysteine-77 of SEQ ID NO:4, cysteine-129 to cysteine-134 of SEQ ID NO:29, and cysteine-94 to cysteine-99 of the human IL-17 amino acid sequence (SEQ ID NO:5); (c) a predicted LVLRRXP domain (where X represents any amino acid) located at about amino acids leucine-46 to proline-52 of SEQ ID NO:2, valine-99 to proline-105 of SEQ ID NO:4, leucine-156 to proline-162 of SEQ ID NO:29, and leucine-120 to proline-126 of the human IL-17 amino acid sequence (SEQ ID NO:5); and (d) a predicted VXVGCTCV domain (where X represents any amino acid) located at about amino acids valine-75 to valine-82 of SEQ ID NO:2, isoleucine-121 to valine-128 of SEQ ID NO:4, valine-187 to valine-192 of SEQ ID NO:29, and valine-140 to valine-147 of the human IL-17 amino acid sequence (SEQ ID NO:5). In addition, the full-length IL-21 molecule shown in Figures 6A and 6B (SEQ ID NO:29) exhibits several additional conserved domains when compared with IL-20 and the other members of the IL-17 family as shown in Figures 3A and 3B). These conserved Domains are underlined in Figures 6A and 6B and are labeled as conserved Domains V, VI, and VII. Specifically, the molecules of the present invention, in particular, SEQ ID NO:29, share a high degree of sequence identity with IL-20, IL-17, mL-17, and vIL-17 in the following conserved domains: (a) a predicted PXCXSAE domain (where X represents any amino acid) located at about amino acids proline-34 to glutamic acid-40 of SEQ ID NO:29; (b) a predicted PXXLVS domain (where X represents any amino acid) located at about amino acids proline-63 to serine-68 of SEQ ID NO:29; and (c) a predicted RSXSPW domain (where X represents any amino acid) located at about amino acids arginine-104 to tryptophan-109 of SEQ ID NO:29. These polypeptide fragments of IL-21 and IL-22 are specifically contemplated in the present invention. Because each of these IL-17 and IL-17-like molecules is thought to be important immunoregulatory molecules, the homology between these IL-17 and IL-17-like molecules and IL-21 and IL-22 suggests that IL-21 and IL-22 may also be important immunoregulatory molecules.

Moreover, based on their apparent sequence identities with IL-17 and IL-20 (see Figures 3A and 3B), the full-length IL-21 and IL-22 polypeptides are each likely to have an amino terminal secretory signal peptide leader sequence. Since the present invention appears to be partial cDNA clones of the IL-21 (SEQ ID NOs:1 and 2) and IL-22 (SEQ ID NOs:3 and 4) molecules (in addition to the full-length IL-21 molecule

shown as SEQ ID NOs:28 and 29), it is also contemplated that the translation products of SEQ ID NOs:2 and 4 of the present invention will be caused to enter the cellular secretory pathway by virtue of being expressed as a fusion proteins comprising several different portions of the N-terminus of the IL-20 molecule of copending U.S. Provisional Application Serial No. 60/060,140 fused to the known coding sequence of the IL-21 or IL-22 molecules of the present invention. Such expression constructs will secrete hybrid IL-20/IL-21 or IL-20/IL-22 molecules from the host cell. The mature IL-21 protein used in these fusion proteins encompasses about amino acids 1-145, while the IL-20/21 fusion protein encompasses about the 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-145 of the IL-21 of SEQ ID NO:2. These polypeptide fragments of IL-21 are specifically contemplated in the present invention. In addition, the mature IL-22 protein used to generate these fusion proteins encompasses about amino acids 1-160, while the IL-20/22 fusion protein encompasses about the 95, 104 or 113 N-terminal amino acids of IL-20 encoded in frame with about amino acids 1-160 of the IL-22 of SEQ ID NO:4. These polypeptide fragments of IL-22 are specifically contemplated in the present invention.

The IL-21 and IL-22 nucleotide sequences identified as SEQ ID NO:1 and SEQ ID NO:3, respectively, were assembled from partially homologous ("overlapping") sequences obtained from the deposited clones. The IL-21 nucleotide sequence identified as SEQ ID NO:28 was assembled from partially homologous ("overlapping") sequences obtained from the deposited clone and a genomic DNA clone. The overlapping sequences specific to the partial IL-21 and IL-22 molecules of the invention and the full-length IL-21 molecule of the invention were each assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in three final sequences identified as SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28.

Therefore, SEQ ID NO:1 and the translated SEQ ID NO:2; SEQ ID NO:3 and the translated SEQ ID NO:4; and SEQ ID NO:28 and the translated SEQ ID NO:29, are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28 are useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1, SEQ ID NO:3, and SEQ ID NO:28, or the cDNA contained in the respective deposited cDNA clones. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 and SEQ ID NO:29 may be used to generate antibodies which bind

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specifically to IL-21 and polypeptides identified from SEQ ID NO:4 may be used to generate antibodies which bind specifically to IL-22.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, and the generated nucleotide sequence identified as SEQ ID NO:28 and the predicted translated amino acid sequence identified as SEQ ID NO:29, but also a sample of plasmid DNA containing a human cDNA of IL-21 deposited with the ATCC. In addition, the present invention also provides not only the generated nucleotide sequence identified as SEQ ID NO:3 and the predicted translated amino acid sequence identified as SEQ ID NO:4, but also a sample of plasmid DNA containing a human cDNA of IL-22 deposited with the ATCC. Accordingly, the nucleotide sequence of the deposited IL-21 and IL-22 clones can be readily determined by sequencing the deposited clone in accordance with known methods. The predicted IL-21 and IL-22 amino acid sequences can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human IL-21 or IL-22 cDNAs, collecting the protein, and determining its sequence.

The present invention also relates to the IL-21 gene corresponding to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:28, SEQ ID NO:29 or the deposited clone which encodes a partial IL-21. The present invention further relates to the IL-22 gene corresponding to SEQ ID NO:3, SEQ ID NO:4, or the deposited clone which encodes IL-22. The IL-21 and IL-22 genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequences and identifying or amplifying the IL-21 and IL-22 genes from appropriate sources of genomic material.

Also provided in the present invention are species homologs of IL-21 and IL-22. Species homologs may be isolated and identified by making suitable probes or



primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homolog.

5 The IL-21 and IL-22 polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

10 The IL-21 and IL-22 polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein. It is often advantageous to include an additional amino acids which comprise secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

15 IL-21 and IL-22 polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of an IL-21 or IL-22 polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in the publication by Smith and Johnson (*Gene* 67:31-40 (1988)). IL-21 and IL-22 polypeptides also can be purified from natural or recombinant sources using antibodies of the invention raised against the IL-21 and 20 IL-22 proteins, respectively, in methods which are well known in the art.

#### Polynucleotide and Polypeptide Variants

"Variant" refers to a polynucleotide or polypeptide differing from the IL-21 and IL-22 polynucleotides or polypeptides; but retaining essential properties thereof. 25 Generally, variants are overall closely similar, and, in many regions, identical to the IL-21 and IL-22 polynucleotide or polypeptide.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 30 100 nucleotides of the reference nucleotide sequence encoding the IL-21 or IL-22 polypeptides. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be inserted, deleted or substituted with another nucleotide. 35 The query sequence may be an entire sequence shown of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:28, the ORF (open reading frame) of either IL-21 or IL-22, or any fragment specified as described herein.

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As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from) a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting (uridine residues (U) to thymidine residues (T). The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, but not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB algorithm does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence ((number of bases at the 5' and 3' ends not matched)/(total number of bases in the query sequence)), so 10% is subtracted from the percent identity score calculated by the

FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence which is, at least, for example, 95% "identical" to (or 5% different from) a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (insertions and deletions are collectively referred to as "indels" in the art) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to (or 10%, 5%, 4%, 3%, 2% or 1% different from), for instance, the amino acid sequences shown in SEQ ID NO:2, or that shown in SEQ ID NO:4, or to the amino acid sequence encoded by deposited cDNA clones, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag and colleagues (*Comp. App. Biosci.* 6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence), so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The IL-21 and IL-22 variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants

produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. IL-21 and IL-22 polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring IL-21 and IL-22 variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (*Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the IL-21 and IL-22 polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron and coworkers reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues (*J. Biol. Chem.* **268**:2984-2988 (1993)). Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli, *et al.*, *J. Biotechnol.* **7**:199-216 (1988)).

In the present case, since the IL-21 and IL-22 proteins of the invention are highly related to the Interleukin-17-like polypeptide family, deletions of N-terminal amino acids up to the cysteine at position 19 of SEQ ID NO:2 and up to the cysteine at position 29 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further N-terminal deletions including the cysteine-19 residue in SEQ ID NO:2 and the cysteine-29 residue in SEQ ID NO:4 would not be expected to retain such biological activities because it is likely that these residues are required for forming a disulfide bridge to provide structural stability which is needed for receptor binding and signal transduction.

However, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the N-termini of the respective proteins. Whether a particular polypeptide lacking

N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the cysteine residue at position number 19, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the cysteine residue at position number 29, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^1$ -87 of SEQ ID NO:2, where  $n^1$  is an integer in the range of 1 to 18, and 19 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for the receptor binding activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^2$ -160 of SEQ ID NO:4, where  $n^2$  is an integer in the range of 1 to 28, and 29 is the position of the first residue from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for the receptor binding activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-87, 2-87, 3-87, 4-87, 5-87, 6-87, 7-87, 8-87, 9-87, 10-87, 11-87, 12-87, 13-87, 14-87, 15-87, 16-87, 17-87, 18-87, and 19-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided. The invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-160, 2-160, 3-160, 4-160, 5-160, 6-160, 7-160, 8-160, 9-160, 10-160, 11-160, 12-160, 13-160, 14-160, 15-160, 16-160, 17-160, 18-160, 19-160, 20-160, 21-160, 22-160, 23-160, 24-160, 25-160, 26-160, 27-160, 28-160, and 29-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

In addition, since the IL-21 and IL-22 proteins of the invention are highly related to the IL-17-like polypeptide family, deletions of C-terminal amino acids up to the leucine at position 83 of SEQ ID NO:2 and up to the proline at position 129 of SEQ ID NO:4 may retain some biological activity. Polypeptides having further C-terminal deletions including the leucine residue at position 83 of SEQ ID NO:2 and the proline at position 129 of SEQ ID NO:4 would not be expected to retain such biological activities since these residues are in the beginning of the conserved domain required for biological activities.

However, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:2, up to the leucine residue at position 83 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the proline residues at position 129 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1- $m^1$  of the amino acid sequence in SEQ ID NO:2, where  $m^1$  is any integer in the range of 83 to 87, and residue 82 is the position of the first residue from the C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for activity of the IL-21 protein. In addition, the present invention also provides polypeptides having the amino acid sequence of residues 1- $m^2$  of the amino acid sequence in SEQ ID NO:4, where  $m^2$  is any integer in the range of 129 to 160, and residue 128 is the position of the first residue from the C-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-83, 1-84, 1-85, 1-86, and 1-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

The present invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues 1-129, 1-130, 1-131, 1-132, 1-133, 1-134, 1-135, 1-136, 1-137, 1-138, 1-139, 1-140, 1-141, 1-142, 1-143, 1-144, 1-145, 1-146, 1-147, 1-148, 1-149, 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 1-156, 1-157, 1-158, 1-159, and 1-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described

generally as having residues  $n^1$ - $m^1$  of SEQ ID NO:2, where  $n^1$  and  $m^1$  are integers as described above. Likewise, the invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, which may be described generally as having residues  $n^2$ - $m^2$  of SEQ ID NO:4, where  $n^2$  and  $m^2$  are integers as described above.

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers conducted extensive mutational analysis of human cytokine IL-1a (*J. Biol. Chem.* 268:22105-22111 (1993)). They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]" (see, Abstract). In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

As mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 or IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 or IL-22 proteins are removed from the N-termini of the respective proteins. Whether a particular polypeptide lacking N-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21



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polypeptide shown in SEQ ID NO:2, up to the valine residue at position number 82, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the aspartic acid residue at position number 155, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^3$ -87 of SEQ ID NO:2, where  $n^3$  is an integer in the range of 1 to 82, and 83 is the position of the first residue from the N-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. Likewise, the present invention provides polypeptides comprising the amino acid sequence of residues  $n^4$ -160 of SEQ ID NO:4, where  $n^4$  is an integer in the range of 1 to 155, and 156 is the position of the first residue from the N-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues R-2 to V-87; V-3 to V-87; D-4 to V-87; T-5 to V-87; D-6 to V-87; E-7 to V-87; D-8 to V-87; R-9 to V-87; Y-10 to V-87; P-11 to V-87; Q-12 to V-87; K-13 to V-87; L-14 to V-87; A-15 to V-87; F-16 to V-87; A-17 to V-87; E-18 to V-87; C-19 to V-87; L-20 to V-87; C-21 to V-87; R-22 to V-87; G-23 to V-87; C-24 to V-87; I-25 to V-87; D-26 to V-87; A-27 to V-87; R-28 to V-87; T-29 to V-87; G-30 to V-87; R-31 to V-87; E-32 to V-87; T-33 to V-87; A-34 to V-87; A-35 to V-87; L-36 to V-87; N-37 to V-87; S-38 to V-87; V-39 to V-87; R-40 to V-87; L-41 to V-87; L-42 to V-87; Q-43 to V-87; S-44 to V-87; L-45 to V-87; L-46 to V-87; V-47 to V-87; L-48 to V-87; R-49 to V-87; R-50 to V-87; R-51 to V-87; P-52 to V-87; C-53 to V-87; S-54 to V-87; R-55 to V-87; D-56 to V-87; G-57 to V-87; S-58 to V-87; G-59 to V-87; L-60 to V-87; P-61 to V-87; T-62 to V-87; P-63 to V-87; G-64 to V-87; A-65 to V-87; F-66 to V-87; A-67 to V-87; F-68 to V-87; H-69 to V-87; T-70 to V-87; E-71 to V-87; F-72 to V-87; I-73 to V-87; H-74 to V-87; V-75 to V-87; P-76 to V-87; V-77 to V-87; G-78 to V-87; C-79 to V-87; T-80 to V-87; C-81 to V-87; and V-82 to V-87 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Further, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues S-2 to P-160; A-3 to P-160; R-4 to P-160; A-5 to P-160; R-6 to P-160; A-7 to P-160; V-8 to P-160; L-9 to P-160; S-10 to P-160; A-11 to P-160; F-12 to P-160; H-13 to P-160; H-14 to P-160; T-15 to P-160; L-16 to P-160; Q-17 to P-160; L-18 to P-160; G-19 to P-160; P-20 to P-160; R-21 to P-160; E-22 to P-160; Q-23 to P-160; A-24 to P-160; R-25 to P-160; N-26 to P-160;

A-27 to P-160; S-28 to P-160; C-29 to P-160; P-30 to P-160; A-31 to P-160; G-32 to P-160; G-33 to P-160; R-34 to P-160; P-35 to P-160; A-36 to P-160; D-37 to P-160; R-38 to P-160; R-39 to P-160; F-40 to P-160; R-41 to P-160; P-42 to P-160; P-43 to P-160; T-44 to P-160; N-45 to P-160; L-46 to P-160; R-47 to P-160; S-48 to P-160; V-49 to P-160; S-50 to P-160; P-51 to P-160; W-52 to P-160; A-53 to P-160; Y-54 to P-160; R-55 to P-160; I-56 to P-160; S-57 to P-160; Y-58 to P-160; D-59 to P-160; P-60 to P-160; A-61 to P-160; R-62 to P-160; Y-63 to P-160; P-64 to P-160; R-65 to P-160; Y-66 to P-160; L-67 to P-160; P-68 to P-160; E-69 to P-160; A-70 to P-160; Y-71 to P-160; C-72 to P-160; L-73 to P-160; C-74 to P-160; R-75 to P-160; G-76 to P-160; C-77 to P-160; L-78 to P-160; T-79 to P-160; G-80 to P-160; L-81 to P-160; F-82 to P-160; G-83 to P-160; E-84 to P-160; E-85 to P-160; D-86 to P-160; V-87 to P-160; R-88 to P-160; F-89 to P-160; R-90 to P-160; S-91 to P-160; A-92 to P-160; P-93 to P-160; V-94 to P-160; Y-95 to P-160; M-96 to P-160; P-97 to P-160; T-98 to P-160; V-99 to P-160; V-100 to P-160; L-101 to P-160; R-102 to P-160; R-103 to P-160; T-104 to P-160; P-105 to P-160; A-106 to P-160; C-107 to P-160; A-108 to P-160; G-109 to P-160; G-110 to P-160; R-111 to P-160; S-112 to P-160; V-113 to P-160; Y-114 to P-160; T-115 to P-160; E-116 to P-160; A-117 to P-160; Y-118 to P-160; V-119 to P-160; T-120 to P-160; I-121 to P-160; P-122 to P-160; V-123 to P-160; G-124 to P-160; C-125 to P-160; T-126 to P-160; C-127 to P-160; V-128 to P-160; P-129 to P-160; E-130 to P-160; P-131 to P-160; E-132 to P-160; K-133 to P-160; D-134 to P-160; A-135 to P-160; D-136 to P-160; S-137 to P-160; I-138 to P-160; N-139 to P-160; S-140 to P-160; S-141 to P-160; I-142 to P-160; D-143 to P-160; K-144 to P-160; Q-145 to P-160; G-146 to P-160; A-147 to P-160; K-148 to P-160; L-149 to P-160; L-150 to P-160; L-151 to P-160; G-152 to P-160; P-153 to P-160; N-154 to P-160; and D-155 to P-160 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the complete or mature IL-21 and IL-22 proteins generally will be retained when less than the majority of the residues of the complete or mature IL-21 and IL-22 proteins are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the

IL-21 polypeptide shown in SEQ ID NO:2, up to the aspartic acid residue at position 6 of SEQ ID NO:2, and polynucleotides encoding such polypeptides. In addition, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-22 polypeptide shown in SEQ ID NO:4, up to the arginine residues at position 6 of SEQ ID NO:4. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m<sup>3</sup> of the amino acid sequence in SEQ ID NO:2, where m<sup>3</sup> is any integer in the range of 6 to 87, and residue 5 is the position of the first residue from the C-terminus of the complete IL-21 polypeptide (shown in SEQ ID NO:2) believed to be required for immunogenic activity of the IL-21 protein. In addition, the present invention also provides polypeptides having the amino acid sequence of residues 1-m<sup>4</sup> of the amino acid sequence in SEQ ID NO:4, where m<sup>4</sup> is any integer in the range of 6 to 160, and residue 5 is the position of the first residue from the C-terminus of the complete IL-22 polypeptide (shown in SEQ ID NO:4) believed to be required for immunogenic activity of the IL-22 protein.

More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues A-1 to S-86; A-1 to R-85; A-1 to P-84; A-1 to L-83; A-1 to V-82; A-1 to C-81; A-1 to T-80; A-1 to C-79; A-1 to G-78; A-1 to V-77; A-1 to P-76; A-1 to V-75; A-1 to H-74; A-1 to I-73; A-1 to F-72; A-1 to E-71; A-1 to T-70; A-1 to H-69; A-1 to F-68; A-1 to A-67; A-1 to F-66; A-1 to A-65; A-1 to G-64; A-1 to P-63; A-1 to T-62; A-1 to P-61; A-1 to L-60; A-1 to G-59; A-1 to S-58; A-1 to G-57; A-1 to D-56; A-1 to R-55; A-1 to S-54; A-1 to C-53; A-1 to P-52; A-1 to R-51; A-1 to R-50; A-1 to R-49; A-1 to L-48; A-1 to V-47; A-1 to L-46; A-1 to L-45; A-1 to S-44; A-1 to Q-43; A-1 to L-42; A-1 to L-41; A-1 to R-40; A-1 to V-39; A-1 to S-38; A-1 to N-37; A-1 to L-36; A-1 to A-35; A-1 to A-34; A-1 to T-33; A-1 to E-32; A-1 to R-31; A-1 to G-30; A-1 to T-29; A-1 to R-28; A-1 to A-27; A-1 to D-26; A-1 to I-25; A-1 to C-24; A-1 to G-23; A-1 to R-22; A-1 to C-21; A-1 to L-20; A-1 to C-19; A-1 to E-18; A-1 to A-17; A-1 to F-16; A-1 to A-15; A-1 to L-14; A-1 to K-13; A-1 to Q-12; A-1 to P-11; A-1 to Y-10; A-1 to R-9; A-1 to D-8; A-1 to E-7; and A-1 to D-6 of SEQ ID NO:2. Polynucleotides encoding these polypeptides also are provided.

Moreover, the invention also provides polynucleotides encoding polypeptides having the amino acid sequence of residues N-1 to G-159; N-1 to A-158; N-1 to P-157; N-1 to A-156; N-1 to D-155; N-1 to N-154; N-1 to P-153; N-1 to G-152; N-1 to L-151; N-1 to L-150; N-1 to L-149; N-1 to K-148; N-1 to A-147; N-1 to G-146; N-1 to Q-145; N-1 to K-144; N-1 to D-143; N-1 to I-142; N-1 to S-141; N-1 to S-140; N-1 to N-139; N-1 to I-138; N-1 to S-137; N-1 to D-136; N-1 to A-135; N-1 to D-134; N-1 to K-133; N-1 to E-132; N-1 to P-131; N-1 to E-130; N-1 to P-129; N-1 to V-128; N-1

to C-127; N-1 to T-126; N-1 to C-125; N-1 to G-124; N-1 to V-123; N-1 to P-122; N-1 to I-121; N-1 to T-120; N-1 to V-119; N-1 to Y-118; N-1 to A-117; N-1 to E-116; N-1 to T-115; N-1 to Y-114; N-1 to V-113; N-1 to S-112; N-1 to R-111; N-1 to G-110; N-1 to G-109; N-1 to A-108; N-1 to C-107; N-1 to A-106; N-1 to P-105; N-1 to T-104; N-1 to R-103; N-1 to R-102; N-1 to L-101; N-1 to V-100; N-1 to V-99; N-1 to T-98; N-1 to P-97; N-1 to M-96; N-1 to Y-95; N-1 to V-94; N-1 to P-93; N-1 to A-92; N-1 to S-91; N-1 to R-90; N-1 to F-89; N-1 to R-88; N-1 to V-87; N-1 to D-86; N-1 to E-85; N-1 to E-84; N-1 to G-83; N-1 to F-82; N-1 to L-81; N-1 to G-80; N-1 to T-79; N-1 to L-78; N-1 to C-77; N-1 to G-76; N-1 to R-75; N-1 to C-74; N-1 to L-73; N-1 to C-72; N-1 to Y-71; N-1 to A-70; N-1 to E-69; N-1 to P-68; N-1 to L-67; N-1 to Y-66; N-1 to R-65; N-1 to P-64; N-1 to Y-63; N-1 to R-62; N-1 to A-61; N-1 to P-60; N-1 to D-59; N-1 to Y-58; N-1 to S-57; N-1 to I-56; N-1 to R-55; N-1 to Y-54; N-1 to A-53; N-1 to W-52; N-1 to P-51; N-1 to S-50; N-1 to V-49; N-1 to S-48; N-1 to R-47; N-1 to L-46; N-1 to N-45; N-1 to T-44; N-1 to P-43; N-1 to P-42; N-1 to R-41; N-1 to F-40; N-1 to R-39; N-1 to R-38; N-1 to D-37; N-1 to A-36; N-1 to P-35; N-1 to R-34; N-1 to G-33; N-1 to G-32; N-1 to A-31; N-1 to P-30; N-1 to C-29; N-1 to S-28; N-1 to A-27; N-1 to N-26; N-1 to R-25; N-1 to A-24; N-1 to Q-23; N-1 to E-22; N-1 to R-21; N-1 to P-20; N-1 to G-19; N-1 to L-18; N-1 to Q-17; N-1 to L-16; N-1 to T-15; N-1 to H-14; N-1 to H-13; N-1 to F-12; N-1 to A-11; N-1 to S-10; N-1 to L-9; N-1 to V-8; N-1 to A-7; and N-1 to R-6 of SEQ ID NO:4. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described generally as having residues  $n^3$ - $m^3$  of SEQ ID NO:2, where  $n^3$  and  $m^3$  are integers as described above. Likewise, the invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, which may be described generally as having residues  $n^4$ - $m^4$  of SEQ ID NO:4, where  $n^4$  and  $m^4$  are integers as described above.

Moreover, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22, described specifically as having residues  $n^4$ - $m^4$  of SEQ ID NO:4 (where  $n^4$  and  $m^4$  are integers as described above) may be excluded from the invention. In particular, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-22 and which is defined by residues  $n^4$ - $m^4$  of SEQ ID NO:4, where  $n^4$  is equal to 21, 22, 23, 24 or 25 and  $m^4$  is equal to 271, 272, 273, 274, 275 or 276 may be excluded from the invention.

Also as mentioned above, even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological

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functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened protein to induce and/or bind to antibodies which recognize the full-length or mature IL-21 polypeptides generally will be retained when less than the majority of the residues of the full-length or mature IL-21 polypeptides are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete or full-length polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:29, up to the valine residue at position number 192, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides comprising the amino acid sequence of residues n<sup>5</sup>-197 of SEQ ID NO:29, where n<sup>5</sup> is an integer in the range of 1 to 192, and 193 is the position of the first residue from the N-terminus of the full-length IL-21 polypeptide (shown in SEQ ID NO:29) believed to be required for immunogenic activity of the IL-21 protein.

More in particular, the invention provides polynucleotides encoding polypeptides comprising the amino acid sequence of residues T-2 to V-197; L-3 to V-197; L-4 to V-197; P-5 to V-197; G-6 to V-197; L-7 to V-197; L-8 to V-197; F-9 to V-197; L-10 to V-197; T-11 to V-197; W-12 to V-197; L-13 to V-197; H-14 to V-197; T-15 to V-197; C-16 to V-197; L-17 to V-197; A-18 to V-197; H-19 to V-197; H-20 to V-197; D-21 to V-197; P-22 to V-197; S-23 to V-197; L-24 to V-197; R-25 to V-197; G-26 to V-197; H-27 to V-197; P-28 to V-197; H-29 to V-197; S-30 to V-197; H-31 to V-197; G-32 to V-197; T-33 to V-197; P-34 to V-197; H-35 to V-197; C-36 to V-197; Y-37 to V-197; S-38 to V-197; A-39 to V-197; E-40 to V-197; E-41 to V-197; L-42 to V-197; P-43 to V-197; L-44 to V-197; G-45 to V-197; Q-46 to V-197; A-47 to V-197; P-48 to V-197; P-49 to V-197; H-50 to V-197; L-51 to V-197; L-52 to V-197; A-53 to V-197; R-54 to V-197; G-55 to V-197; A-56 to V-197; K-57 to V-197; W-58 to V-197; G-59 to V-197; Q-60 to V-197; A-61 to V-197; L-62 to V-197; P-63 to V-197; V-64 to V-197; A-65 to V-197; L-66 to V-197; V-67 to V-197; S-68 to V-197; S-69 to V-197; L-70 to V-197; E-71 to V-197; A-72 to V-197; A-73 to V-197; S-74 to V-197; H-75 to V-197; R-76 to V-197; G-77 to V-197; R-78 to V-197; H-79 to V-197; E-80 to V-197; R-81 to V-197; P-82 to V-197; S-83 to V-197; A-84 to V-197; T-85 to V-197; T-86 to V-197; Q-87 to V-197; C-88 to V-197; P-89 to V-197; V-90 to V-197; L-91 to V-197; R-92 to V-197; P-93 to V-197; E-94 to V-197; E-95 to V-197; V-96 to V-197; L-97 to V-197; E-98 to V-197; A-99 to V-197; D-100 to V-197; T-101 to V-197; H-102 to V-197; Q-103 to V-197; R-104 to V-197; S-105 to V-197; I-106 to V-197; S-107 to V-197; P-108 to V-197; W-109 to V-197; R-110 to V-197; Y-111 to V-197; R-112 to

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V-197; V-113 to V-197; D-114 to V-197; T-115 to V-197; D-116 to V-197; E-117 to V-197; D-118 to V-197; R-119 to V-197; Y-120 to V-197; P-121 to V-197; Q-122 to V-197; K-123 to V-197; L-124 to V-197; A-125 to V-197; F-126 to V-197; A-127 to V-197; E-128 to V-197; C-129 to V-197; L-130 to V-197; C-131 to V-197; R-132 to V-197; G-133 to V-197; C-134 to V-197; I-135 to V-197; D-136 to V-197; A-137 to V-197; R-138 to V-197; T-139 to V-197; G-140 to V-197; R-141 to V-197; E-142 to V-197; T-143 to V-197; A-144 to V-197; A-145 to V-197; L-146 to V-197; N-147 to V-197; S-148 to V-197; V-149 to V-197; R-150 to V-197; L-151 to V-197; L-152 to V-197; Q-153 to V-197; S-154 to V-197; L-155 to V-197; L-156 to V-197; V-157 to V-197; L-158 to V-197; R-159 to V-197; R-160 to V-197; R-161 to V-197; P-162 to V-197; C-163 to V-197; S-164 to V-197; R-165 to V-197; D-166 to V-197; G-167 to V-197; S-168 to V-197; G-169 to V-197; L-170 to V-197; P-171 to V-197; T-172 to V-197; P-173 to V-197; G-174 to V-197; A-175 to V-197; F-176 to V-197; A-177 to V-197; F-178 to V-197; H-179 to V-197; T-180 to V-197; E-181 to V-197; F-182 to V-197; I-183 to V-197; H-184 to V-197; V-185 to V-197; P-186 to V-197; V-187 to V-197; G-188 to V-197; C-189 to V-197; T-190 to V-197; C-191 to V-197; and V-192 to V-197 of SEQ ID NO:29. Polynucleotides encoding these polypeptides also are provided.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other biological activities may still be retained. Thus, the ability of the shortened polypeptide to induce and/or bind to antibodies which recognize the full-length or mature IL-21 polypeptide generally will be retained when less than the majority of the residues of the full-length or mature IL-21 polypeptides are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete protein retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art.

Accordingly, the present invention further provides polypeptides having one or more residues removed from the carboxy terminus of the amino acid sequence of the IL-21 polypeptide shown in SEQ ID NO:29, up to the glycine residue at position 6 of SEQ ID NO:29, and polynucleotides encoding such polypeptides. In particular, the present invention provides polypeptides having the amino acid sequence of residues 1-m<sup>5</sup> of the amino acid sequence in SEQ ID NO:29, where m<sup>5</sup> is any integer in the range of 6 to 196, and residue 5 is the position of the first residue from the C-terminus of the full-length IL-21 polypeptide (shown in SEQ ID NO:29) believed to be required for immunogenic activity of the IL-21 protein.

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More in particular, the invention provides polynucleotides encoding polypeptides having the amino acid sequence of residues M-1 to S-196; M-1 to R-195; M-1 to P-194; M-1 to L-193; M-1 to V-192; M-1 to C-191; M-1 to T-190; M-1 to C-189; M-1 to G-188; M-1 to V-187; M-1 to P-186; M-1 to V-185; M-1 to H-184; M-1 to I-183; M-1 to F-182; M-1 to E-181; M-1 to T-180; M-1 to H-179; M-1 to F-178; M-1 to A-177; M-1 to F-176; M-1 to A-175; M-1 to G-174; M-1 to P-173; M-1 to T-172; M-1 to P-171; M-1 to L-170; M-1 to G-169; M-1 to S-168; M-1 to G-167; M-1 to D-166; M-1 to R-165; M-1 to S-164; M-1 to C-163; M-1 to P-162; M-1 to R-161; M-1 to R-160; M-1 to R-159; M-1 to L-158; M-1 to V-157; M-1 to L-156; M-1 to L-155; M-1 to S-154; M-1 to Q-153; M-1 to L-152; M-1 to L-151; M-1 to R-150; M-1 to V-149; M-1 to S-148; M-1 to N-147; M-1 to L-146; M-1 to A-145; M-1 to A-144; M-1 to T-143; M-1 to E-142; M-1 to R-141; M-1 to G-140; M-1 to T-139; M-1 to R-138; M-1 to A-137; M-1 to D-136; M-1 to I-135; M-1 to C-134; M-1 to G-133; M-1 to R-132; M-1 to C-131; M-1 to L-130; M-1 to C-129; M-1 to E-128; M-1 to A-127; M-1 to F-126; M-1 to A-125; M-1 to L-124; M-1 to K-123; M-1 to Q-122; M-1 to P-121; M-1 to Y-120; M-1 to R-119; M-1 to D-118; M-1 to E-117; M-1 to D-116; M-1 to T-115; M-1 to D-114; M-1 to V-113; M-1 to R-112; M-1 to Y-111; M-1 to R-110; M-1 to W-109; M-1 to P-108; M-1 to S-107; M-1 to I-106; M-1 to S-105; M-1 to R-104; M-1 to Q-103; M-1 to H-102; M-1 to T-101; M-1 to D-100; M-1 to A-99; M-1 to E-98; M-1 to L-97; M-1 to V-96; M-1 to E-95; M-1 to E-94; M-1 to P-93; M-1 to R-92; M-1 to L-91; M-1 to V-90; M-1 to P-89; M-1 to C-88; M-1 to Q-87; M-1 to T-86; M-1 to T-85; M-1 to A-84; M-1 to S-83; M-1 to P-82; M-1 to R-81; M-1 to E-80; M-1 to H-79; M-1 to R-78; M-1 to G-77; M-1 to R-76; M-1 to H-75; M-1 to S-74; M-1 to A-73; M-1 to A-72; M-1 to E-71; M-1 to L-70; M-1 to S-69; M-1 to S-68; M-1 to V-67; M-1 to L-66; M-1 to A-65; M-1 to V-64; M-1 to P-63; M-1 to L-62; M-1 to A-61; M-1 to Q-60; M-1 to G-59; M-1 to W-58; M-1 to K-57; M-1 to A-56; M-1 to G-55; M-1 to R-54; M-1 to A-53; M-1 to L-52; M-1 to L-51; M-1 to H-50; M-1 to P-49; M-1 to P-48; M-1 to A-47; M-1 to Q-46; M-1 to G-45; M-1 to L-44; M-1 to P-43; M-1 to L-42; M-1 to E-41; M-1 to E-40; M-1 to A-39; M-1 to S-38; M-1 to Y-37; M-1 to C-36; M-1 to H-35; M-1 to P-34; M-1 to T-33; M-1 to G-32; M-1 to H-31; M-1 to S-30; M-1 to H-29; M-1 to P-28; M-1 to H-27; M-1 to G-26; M-1 to R-25; M-1 to L-24; M-1 to S-23; M-1 to P-22; M-1 to D-21; M-1 to H-20; M-1 to H-19; M-1 to A-18; M-1 to L-17; M-1 to C-16; M-1 to T-15; M-1 to H-14; M-1 to L-13; M-1 to W-12; M-1 to T-11; M-1 to L-10; M-1 to F-9; M-1 to L-8; M-1 to L-7; and M-1 to G-6 of SEQ ID NO:29. Polynucleotides encoding these polypeptides also are provided.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, which may be described

generally as having residues  $n^5$ - $m^5$  of SEQ ID NO:29, where  $n^5$  and  $m^5$  are integers as described above.

Moreover, any polypeptide having one or more amino acids deleted from both the amino and the carboxyl termini of IL-21, described specifically as having residues  $n^5$ - $m^5$  of SEQ ID NO:29 (where  $n^5$  and  $m^5$  are integers as described above) may be excluded from the invention.

The invention further includes IL-21 and IL-22 polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided by Bowie and colleagues (*Science* 247:1306-1310 (1990)), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of an aliphatic or hydrophobic amino acid with another aliphatic or hydrophobic amino acid such as Ala, Val, Leu or Ile; replacement of a hydroxyl residue with another hydroxyl residue such as Ser or Thr; replacement of an acidic residue with another acidic residue such as Asp or Glu; replacement of an amide residue with another amide residue such as Asn or Gln; replacement of a basic residue with another basic



residue such as Lys, Arg, or His; replacement of an aromatic residue with another aromatic residue such as Phe, Tyr, or Trp, and replacement of a small-sized amino acid with another small-sized residue such as Ala, Ser, Thr, Met, or Gly.

Besides conservative amino acid substitution, variants of IL-21 and IL-22 include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, IL-21 and IL-22 polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (Pinckard, *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins, *et al.*, *Diabetes* 36:838-845 (1987); Cleland, *et al.*, *Crit. Rev. Ther. Drug Carrier Systems* 10:307-377 (1993)).

#### Polynucleotide and Polypeptide Fragments

The invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:3 which have been determined from the following related cDNA clones: HE2CD08R (SEQ ID NO:24); HAGBX04R (SEQ ID NO:25); HCEBA24FB (SEQ ID NO:26); and HCELE59R (SEQ ID NO:27). Furthermore, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:28 which has been determined from a related cDNA clone designated HTGED19RB (SEQ ID NO:30). Such polynucleotides (i.e., SEQ ID NOs:24, 25, 26, and 30) may preferably be excluded from the present invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clones or shown in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:28. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20

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or more contiguous bases from the cDNA sequence contained in the deposited clones or the nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:28. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of IL-21 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, or 701 to the end of SEQ ID NO:1 or the cDNA contained in the deposited clone. In addition, representative examples of IL-22 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1551-1600, or 1601 to the end of SEQ ID NO:3 or the cDNA contained in the deposited clone. Moreover, representative examples of the full-length IL-21 polynucleotide fragments include, for example, fragments having a sequence from about nucleotide number 1-1025, 50-1025, 100-1025, 150-1025, 200-1025, 250-1025, 300-1025, 350-1025, 400-1025, 450-1025, 500-1025, 550-1025, 600-1025, 650-1025, 700-1025, 750-1025, 800-1025, 850-1025, 900-1025, 950-1025, 1000-1025, 1-1000, 50-1000, 100-1000, 150-1000, 200-1000, 250-1000, 300-1000, 350-1000, 400-1000, 450-1000, 500-1000, 550-1000, 600-1000, 650-1000, 700-1000, 750-1000, 800-1000, 850-1000, 900-1000, 950-1000, 1-950, 50-950, 100-950, 150-950, 200-950, 250-950, 300-950, 350-950, 400-950, 450-950, 500-950, 550-950, 600-950, 650-950, 700-950, 750-950, 800-950, 850-950, 900-950, 1-900, 50-900, 100-900, 150-900, 200-900, 250-900, 300-900, 350-900, 400-900, 450-900, 500-900, 550-900, 600-900, 650-900, 700-900, 750-900, 800-900, 850-900, 1-850, 50-850, 100-850, 150-850, 200-850, 250-850, 300-850, 350-850, 400-850, 450-850, 500-850, 550-850, 600-850, 650-850, 700-850, 750-850, 800-850, 1-800, 50-800, 100-800, 150-800, 200-800, 250-800, 300-800, 350-800, 400-800, 450-800, 500-800, 550-800, 600-800, 650-800, 700-800, 750-800, 1-750, 50-750, 100-750, 150-750, 200-750, 250-750, 300-750, 350-750, 400-750, 450-750, 500-750, 550-750, 600-750, 650-750, 700-750, 1-700, 50-700, 100-700, 150-700, 200-700, 250-700, 300-700, 350-700, 400-700, 450-700, 500-700, 550-700, 600-700, 650-700, 1-650, 50-650, 100-650, 150-650, 200-650, 250-650, 300-650, 350-650, 400-650, 450-650, 500-650, 550-650, 600-650, 1-600, 50-600, 100-600, 150-600,

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200-600, 250-600, 300-600, 350-600, 400-600, 450-600, 500-600, 550-600, 1-550, 50-550, 100-550, 150-550, 200-550, 250-550, 300-550, 350-550, 400-550, 450-550, 500-550, 1-500, 50-500, 100-500, 150-500, 200-500, 250-500, 300-500, 350-500, 400-500, 450-500, 1-450, 50-450, 100-450, 150-450, 200-450, 250-450, 300-450, 350-450, 400-450, 1-400, 50-400, 100-400, 150-400, 200-400, 250-400, 300-400, 350-400, 1-350, 50-350, 100-350, 150-350, 200-350, 250-350, 300-350, 1-300, 50-300, 100-300, 150-300, 200-300, 250-300, 1-250, 50-250, 100-250, 150-250, 200-250, 1-200, 50-200, 100-200, 150-200, 1-150, 50-150, 100-150, 1-100, 50-100, and 1-50 of SEQ ID NO:28. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

Further, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:1 from residue 1 to 650, 25 to 650, 50 to 650, 75 to 650, 100 to 650, 125 to 650, 150 to 650, 175 to 650, 200 to 650, 225 to 650, 250 to 650, 275 to 650, 300 to 650, 325 to 650, 350 to 650, 375 to 650, 400 to 650, 425 to 650, 500 to 650, 525 to 650, 550 to 650, 575 to 650, 600 to 650, 625 to 650, 1 to 600, 25 to 600, 50 to 600, 75 to 600, 100 to 600, 125 to 600, 150 to 600, 175 to 600, 200 to 600, 225 to 600, 250 to 600, 275 to 600, 300 to 600, 325 to 600, 350 to 600, 375 to 600, 400 to 600, 425 to 600, 500 to 600, 525 to 600, 550 to 600, 575 to 600, 1 to 550, 25 to 550, 50 to 550, 75 to 550, 100 to 550, 125 to 550, 150 to 550, 175 to 550, 200 to 550, 225 to 550, 250 to 550, 275 to 550, 300 to 550, 325 to 550, 350 to 550, 375 to 550, 400 to 550, 425 to 550, 500 to 550, 525 to 550, 1 to 500, 25 to 500, 50 to 500, 75 to 500, 100 to 500, 125 to 500, 150 to 500, 175 to 500, 200 to 500, 225 to 500, 250 to 500, 275 to 500, 300 to 500, 325 to 500, 350 to 500, 375 to 500, 400 to 500, 425 to 500, 450 to 500, 475 to 500, 1 to 450, 25 to 450, 50 to 450, 75 to 450, 100 to 450, 125 to 450, 150 to 450, 175 to 450, 200 to 450, 225 to 450, 250 to 450, 275 to 450, 300 to 450, 325 to 450, 350 to 450, 375 to 450, 400 to 450, 425 to 450, 1 to 400, 25 to 400, 50 to 400, 75 to 400, 100 to 400, 125 to 400, 150 to 400, 175 to 400, 200 to 400, 225 to 400, 250 to 400, 275 to 400, 300 to 400, 325 to 400, 350 to 400, 375 to 400, 1 to 350, 25 to 350, 50 to 350, 75 to 350, 100 to 350, 125 to 350, 150 to 350, 175 to 350, 200 to 350, 225 to 350, 250 to 350, 275 to 350, 300 to 350, 325 to 350, 1 to 300, 25 to 300, 50 to 300, 75 to 300, 100 to 300, 125 to 300, 150 to 300, 175 to 300, 200 to 300, 225 to 300, 250 to 300, 275 to 300, 1 to 250, 25 to 250, 50 to 250, 75 to 250, 100 to 250, 125 to 250, 150 to 250, 175 to 250, 200 to 250, 225 to 250, 1 to 200, 25 to 200, 50 to 200, 75 to 200, 100 to 200, 125 to 200, 150 to 200, 175 to 200, 1 to 150, 25 to 150,

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50 to 150, 75 to 150, 100 to 150, 125 to 150, 1 to 100, 25 to 100, 50 to 100, 75 to 100, 1 to 50, and 25 to 50.

Moreover, the invention includes a polynucleotide comprising any portion of at least about 30 nucleotides, preferably at least about 50 nucleotides, of SEQ ID NO:3

5 from residue 300 to 850. More preferably, the invention includes a polynucleotide comprising nucleotide residues 50 to 850, 75 to 850, 100 to 850, 125 to 850, 150 to 850, 175 to 850, 200 to 850, 225 to 850, 250 to 850, 275 to 850, 300 to 850, 325 to 850, 350 to 850, 375 to 850, 400 to 850, 425 to 850, 450 to 850, 475 to 850, 500 to 850, 525 to 850, 550 to 850, 575 to 850, 600 to 850, 625 to 850, 650 to 850, 675 to 850, 700 to 850, 750 to 850, 775 to 850, 800 to 850, 50 to 800, 75 to 800, 100 to 800, 125 to 800, 150 to 800, 175 to 800, 200 to 800, 225 to 800, 250 to 800, 275 to 800, 300 to 800, 325 to 800, 350 to 800, 375 to 800, 400 to 800, 425 to 800, 450 to 800, 475 to 800, 500 to 800, 525 to 800, 550 to 800, 575 to 800, 600 to 800, 625 to 800, 650 to 800, 675 to 800, 700 to 800, 750 to 800, 50 to 750, 75 to 750, 100 to 750, 125 to 750, 150 to 750, 175 to 750, 200 to 750, 225 to 750, 250 to 750, 275 to 750, 300 to 750, 325 to 750, 350 to 750, 375 to 750, 400 to 750, 425 to 750, 450 to 750, 475 to 750, 500 to 750, 525 to 750, 550 to 750, 575 to 750, 600 to 750, 625 to 750, 650 to 750, 675 to 750, 700 to 750, 50 to 700, 75 to 700, 100 to 700, 125 to 700, 150 to 700, 175 to 700, 200 to 700, 225 to 700, 250 to 700, 275 to 700, 300 to 700, 325 to 700, 350 to 700, 375 to 700, 400 to 700, 425 to 700, 450 to 700, 475 to 700, 500 to 700, 525 to 700, 550 to 700, 575 to 700, 600 to 700, 625 to 700, 650 to 700, 50 to 650, 75 to 650, 100 to 650, 125 to 650, 150 to 650, 175 to 650, 200 to 650, 225 to 650, 250 to 650, 275 to 650, 300 to 650, 325 to 650, 350 to 650, 375 to 650, 400 to 650, 425 to 650, 450 to 650, 475 to 650, 500 to 650, 525 to 650, 550 to 650, 575 to 650, 600 to 650, 50 to 600, 75 to 600, 100 to 600, 125 to 600, 150 to 600, 175 to 600, 200 to 600, 225 to 600, 250 to 600, 275 to 600, 300 to 600, 325 to 600, 350 to 600, 375 to 600, 400 to 600, 425 to 600, 450 to 600, 475 to 600, 500 to 600, 525 to 600, 550 to 600, 50 to 550, 75 to 550, 100 to 550, 125 to 550, 150 to 550, 175 to 550, 200 to 550, 225 to 550, 250 to 550, 275 to 550, 300 to 550, 325 to 550, 350 to 550, 375 to 550, 400 to 550, 425 to 550, 450 to 550, 475 to 550, 500 to 550, 50 to 500, 75 to 500, 100 to 500, 125 to 500, 150 to 500, 175 to 500, 200 to 500, 225 to 500, 250 to 500, 275 to 500, 300 to 500, 325 to 500, 350 to 500, 375 to 500, 400 to 500, 425 to 500, 450 to 500, 50 to 450, 75 to 450, 100 to 450, 125 to 450, 150 to 450, 175 to 450, 200 to 450, 225 to 450, 250 to 450, 275 to 450, 300 to 450, 325 to 450, 350 to 450, 375 to 450, 400 to 450, 50 to 400, 75 to 400, 100 to 400, 125 to 400, 150 to 400, 175 to 400, 200 to 400, 225 to 400, 250 to 400, 275 to 400, 300 to 400, 325 to 400, 350 to 400, 50 to 350, 75 to 350, 100 to 350, 125 to

350, 150 to 350, 175 to 350, 200 to 350, 225 to 350, 250 to 350, 275 to 350, 300 to 350, 50 to 300, 75 to 300, 100 to 300, 125 to 300, 150 to 300, 175 to 300, 200 to 300, 225 to 300, and 250 to 300.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:29 or encoded by the cDNAs contained in the deposited clones. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the partial IL-21 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-83 or to the end of the coding region. Moreover, polypeptide fragments of IL-21 can be about 10, 20, 30, 40, 50, 60, 70, or 80 amino acids in length. Representative examples of polypeptide fragments of the IL-22 invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 100-120, 120-140, 140-160, or to the end of the coding region. Moreover, polypeptide fragments of IL-22 can be about 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, or 150 amino acids in length. Representative examples of polypeptide fragments of the full-length IL-21 of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 100-120, 120-140, 140-160, 160-180, 180-200 or 180-to the end of the coding region. Moreover, polypeptide fragments of the full-length IL-21 can be about 10, 20, 30, 40, 50, 60, 70, 80, 100, 120, 140, 150, 160, 170, 180 or 190 amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted IL-21 and IL-22 proteins as well as the mature forms. Further preferred polypeptide fragments include the secreted IL-21 and IL-22 proteins or the mature forms having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted or the mature form of the IL-21 and IL-22 polypeptides. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted or the mature form of the IL-21 and IL-22 polypeptides. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these IL-21 and IL-22 polypeptide fragments are also preferred.

Also preferred are IL-21 and IL-22 polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions,

turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:29 falling within conserved domains are specifically contemplated by the present invention (Figures 4, 5, and 7). Moreover, polynucleotide fragments encoding these domains are also contemplated.

In additional embodiments, the polynucleotides of the invention encode functional attributes of IL-21 or IL-22. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of IL-21 or IL-22.

The data representing the structural or functional attributes of IL-21 set forth in Figure 7 and/or Table I, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. The data representing the structural or functional attributes of IL-22 set forth in Figure 5 and/or Table II, as described above, was generated using the various modules and algorithms of the DNA\*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Tables I and II can be used to determine regions of IL-21 and IL-22, respectively, which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in Figures 5 and 7, but may, as shown in Tables II and I, respectively, be represented or identified by using tabular representations of the data presented in Figures 5 and 7, respectively. The DNA\*STAR computer algorithm used to generate Figures 5 and 7 (set on the original default parameters) was used to present the data in Figures 5 and 7 in a tabular format (See Tables II and I, respectively). The tabular format of the data in Figures 5 and 7 may be used to easily determine specific boundaries of a preferred region.

The above-mentioned preferred regions set out in Figures 5 and 7 and in Tables II and I, respectively, include, but are not limited to, regions of the aforementioned

- types identified by analysis of the amino acid sequence set out in Figures 6A and 6B. As set out in Figure 7 and in Table I, and in Figure 5 and Table II, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions,
- 5 Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index.

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Table I

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  | IX    | X | XI | XII | XIII  | XIV  |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|-------|---|----|-----|-------|------|
| 5  | Met | 1        | A | .  | .   | .  | . | .  | .   | -0.80 | 0.76  | . | .  | .   | -0.40 | 0.36 |
|    | Thr | 2        | . | .  | B   | .  | . | .  | .   | -0.76 | 0.76  | . | .  | .   | -0.40 | 0.44 |
|    | Leu | 3        | A | .  | .   | .  | . | .  | .   | -1.18 | 0.76  | . | .  | .   | -0.40 | 0.34 |
|    | Leu | 4        | A | .  | .   | .  | . | T  | .   | -1.60 | 1.01  | . | .  | .   | -0.20 | 0.28 |
|    | Pro | 5        | A | .  | .   | .  | . | T  | .   | -1.91 | 1.09  | . | F  | .   | -0.05 | 0.16 |
| 10 | Gly | 6        | A | .  | .   | .  | . | T  | .   | -2.12 | 1.39  | . | .  | .   | -0.20 | 0.17 |
|    | Leu | 7        | A | .  | .   | .  | . | T  | .   | -2.12 | 1.39  | . | .  | .   | -0.20 | 0.17 |
|    | Leu | 8        | A | A  | .   | .  | . | .  | .   | -1.60 | 1.19  | . | .  | .   | -0.60 | 0.16 |
|    | Phe | 9        | A | A  | .   | .  | . | .  | .   | -1.60 | 1.67  | . | .  | .   | -0.60 | 0.17 |
|    | Leu | 10       | A | A  | .   | .  | . | .  | .   | -1.42 | 1.93  | . | .  | .   | -0.60 | 0.17 |
| 15 | Thr | 11       | A | A  | .   | .  | . | .  | .   | -1.39 | 1.74  | . | .  | .   | -0.60 | 0.28 |
|    | Trp | 12       | A | A  | .   | .  | . | .  | .   | -1.24 | 1.54  | . | .  | .   | -0.60 | 0.46 |
|    | Leu | 13       | A | A  | .   | .  | . | .  | .   | -1.24 | 1.33  | . | .  | .   | -0.60 | 0.30 |
|    | His | 14       | A | A  | .   | .  | . | .  | .   | -1.13 | 1.33  | * | .  | .   | -0.60 | 0.17 |
|    | Thr | 15       | A | A  | .   | .  | . | .  | .   | -0.36 | 1.34  | . | .  | .   | -0.60 | 0.17 |
| 20 | Cys | 16       | A | A  | .   | .  | . | .  | .   | -0.08 | 0.93  | . | .  | .   | -0.60 | 0.27 |
|    | Leu | 17       | A | A  | .   | .  | . | .  | .   | 0.21  | 0.74  | . | .  | .   | -0.60 | 0.27 |
|    | Ala | 18       | . | A  | .   | .  | T | .  | .   | 0.81  | 0.24  | . | .  | .   | 0.10  | 0.32 |
|    | His | 19       | . | A  | .   | .  | T | .  | .   | 0.54  | 0.19  | . | .  | .   | 0.44  | 0.91 |
|    | His | 20       | . | A  | .   | .  | . | .  | C   | 0.04  | -0.00 | * | .  | .   | 1.33  | 1.48 |
| 25 | Asp | 21       | . | .  | .   | .  | . | T  | C   | 0.82  | -0.00 | * | F  | .   | 2.22  | 1.21 |
|    | Pro | 22       | . | .  | .   | .  | T | T  | .   | 1.29  | -0.50 | * | F  | .   | 2.76  | 1.74 |
|    | Ser | 23       | . | .  | .   | .  | T | T  | .   | 1.84  | -0.57 | * | F  | .   | 3.40  | 1.27 |
|    | Leu | 24       | . | .  | .   | .  | T | T  | .   | 1.67  | -0.57 | * | F  | .   | 3.06  | 1.03 |
|    | Arg | 25       | . | .  | .   | .  | T | .  | .   | 1.67  | -0.14 | * | F  | .   | 2.35  | 1.03 |
| 30 | Gly | 26       | . | .  | .   | .  | T | .  | .   | 1.37  | -0.07 | * | F  | .   | 2.14  | 1.05 |
|    | His | 27       | . | .  | .   | .  | . | T  | C   | 1.54  | -0.07 | * | .  | .   | 1.78  | 1.70 |
|    | Pro | 28       | . | .  | .   | .  | . | T  | C   | 1.50  | -0.26 | * | .  | .   | 1.57  | 1.18 |
|    | His | 29       | . | .  | .   | .  | T | T  | .   | 2.00  | 0.17  | * | .  | .   | 1.30  | 1.18 |
|    | Ser | 30       | . | .  | .   | .  | T | T  | .   | 1.68  | 0.23  | * | .  | .   | 1.17  | 1.26 |
| 35 | His | 31       | . | .  | .   | .  | T | .  | .   | 1.99  | 0.16  | . | .  | .   | 0.84  | 1.26 |
|    | Gly | 32       | . | .  | .   | .  | T | .  | .   | 1.36  | 0.23  | . | F  | .   | 0.86  | 1.26 |
|    | Thr | 33       | . | .  | .   | .  | . | T  | C   | 1.32  | 0.30  | . | F  | .   | 0.58  | 0.50 |
|    | Pro | 34       | . | .  | .   | .  | . | T  | C   | 1.06  | 0.67  | . | F  | .   | 0.15  | 0.58 |
|    | His | 35       | . | .  | .   | .  | T | T  | .   | 0.77  | 0.56  | . | .  | .   | 0.20  | 0.78 |
| 40 | Cys | 36       | . | .  | .   | .  | T | T  | .   | 0.80  | 0.63  | . | .  | .   | 0.20  | 0.55 |
|    | Tyr | 37       | . | A  | .   | .  | T | .  | C   | 1.14  | 0.14  | . | .  | .   | 0.10  | 0.61 |
|    | Ser | 38       | . | A  | .   | .  | . | .  | C   | 0.64  | -0.29 | . | .  | .   | 0.50  | 0.78 |
|    | Ala | 39       | A | A  | .   | .  | . | .  | .   | 0.64  | -0.10 | . | .  | .   | 0.45  | 1.20 |
|    | Glu | 40       | A | A  | .   | .  | . | .  | .   | -0.13 | -0.24 | . | F  | .   | 0.60  | 1.19 |
| 45 | Glu | 41       | A | A  | .   | .  | . | .  | .   | 0.19  | -0.31 | . | .  | .   | 0.30  | 0.73 |
|    | Leu | 42       | A | .  | .   | .  | . | T  | .   | 0.43  | -0.27 | . | .  | .   | 0.70  | 0.72 |
|    | Pro | 43       | A | .  | .   | .  | . | T  | .   | 0.14  | -0.37 | . | .  | .   | 0.70  | 0.72 |
|    | Leu | 44       | . | .  | .   | .  | T | T  | .   | 0.52  | 0.13  | . | .  | .   | 0.50  | 0.42 |
|    | Gly | 45       | . | .  | .   | .  | T | T  | .   | 0.31  | 0.56  | . | F  | .   | 0.35  | 0.78 |
| 50 | Gln | 46       | A | .  | .   | .  | . | .  | .   | 0.28  | 0.30  | . | F  | .   | 0.05  | 0.78 |
|    | Ala | 47       | . | .  | .   | .  | . | .  | C   | 0.28  | 0.37  | * | F  | .   | 0.40  | 1.29 |
|    | Pro | 48       | . | .  | .   | .  | . | T  | C   | -0.32 | 0.37  | * | F  | .   | 0.60  | 1.08 |
|    | Pro | 49       | A | .  | .   | .  | . | T  | .   | -0.10 | 0.63  | * | F  | .   | -0.05 | 0.51 |
|    | His | 50       | A | .  | .   | .  | . | T  | .   | 0.36  | 0.73  | * | .  | .   | -0.20 | 0.51 |
| 55 | Leu | 51       | A | .  | .   | .  | . | T  | .   | 0.01  | 0.23  | * | .  | .   | 0.10  | 0.65 |
|    | Leu | 52       | A | A  | .   | .  | . | .  | .   | 0.01  | 0.23  | * | .  | .   | -0.30 | 0.42 |
|    | Ala | 53       | A | A  | .   | .  | . | .  | .   | 0.27  | 0.30  | * | .  | .   | -0.30 | 0.31 |
|    | Arg | 54       | A | A  | .   | .  | . | .  | .   | 0.19  | -0.20 | * | .  | .   | 0.30  | 0.75 |
|    | Gly | 55       | A | A  | .   | .  | . | .  | .   | -0.12 | 0.03  | * | F  | .   | -0.15 | 0.95 |
| 60 | Ala | 56       | A | A  | .   | .  | . | .  | .   | 0.69  | -0.23 | * | F  | .   | 0.45  | 0.93 |



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Table I (continued)

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  | IX    | X | XI | XII | XIII  | XIV  |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|-------|---|----|-----|-------|------|
| 5  | Lys | 57       | . | A  | .   | .  | T | .  | .   | 0.91  | -0.33 | * | .  | F   | 0.85  | 0.83 |
|    | Trp | 58       | . | A  | .   | .  | T | .  | .   | 0.69  | 0.17  | * | .  | F   | 0.25  | 0.84 |
|    | Gly | 59       | . | A  | .   | .  | . | .  | C   | 0.37  | 0.43  | * | .  | F   | -0.25 | 0.69 |
|    | Gln | 60       | A | A  | .   | .  | . | .  | .   | -0.14 | 0.36  | * | .  | .   | -0.30 | 0.53 |
|    | Ala | 61       | . | A  | .   | .  | . | .  | C   | -0.14 | 1.00  | * | .  | .   | -0.40 | 0.38 |
| 10 | Leu | 62       | . | A  | B   | .  | . | .  | .   | -1.00 | 0.59  | * | .  | .   | -0.60 | 0.38 |
|    | Pro | 63       | . | A  | B   | .  | . | .  | .   | -1.57 | 0.84  | . | .  | .   | -0.60 | 0.18 |
|    | Val | 64       | A | A  | .   | .  | . | .  | .   | -1.52 | 1.09  | . | .  | .   | -0.60 | 0.13 |
|    | Ala | 65       | A | A  | .   | .  | . | .  | .   | -1.82 | 0.97  | . | .  | .   | -0.60 | 0.22 |
|    | Leu | 66       | A | A  | .   | .  | . | .  | .   | -2.04 | 0.67  | . | .  | .   | -0.60 | 0.19 |
| 15 | Val | 67       | A | A  | .   | .  | . | .  | .   | -1.23 | 0.93  | . | .  | .   | -0.60 | 0.21 |
|    | Ser | 68       | A | A  | .   | .  | . | .  | .   | -1.61 | 0.29  | . | .  | .   | -0.30 | 0.36 |
|    | Ser | 69       | A | A  | .   | .  | . | .  | .   | -1.34 | 0.29  | . | .  | .   | -0.30 | 0.44 |
|    | Leu | 70       | A | A  | .   | .  | . | .  | .   | -1.06 | 0.10  | * | .  | .   | -0.30 | 0.60 |
|    | Glu | 71       | A | A  | .   | .  | . | .  | .   | -0.28 | -0.16 | * | .  | .   | 0.30  | 0.60 |
| 20 | Ala | 72       | A | A  | .   | .  | . | .  | .   | 0.69  | -0.04 | * | .  | .   | 0.30  | 0.61 |
|    | Ala | 73       | A | A  | .   | .  | . | .  | .   | 0.64  | -0.43 | * | *  | .   | 0.79  | 1.45 |
|    | Ser | 74       | A | .  | .   | .  | . | .  | .   | 1.06  | -0.69 | * | *  | .   | 1.48  | 0.83 |
|    | His | 75       | A | .  | .   | .  | . | T  | .   | 1.83  | -0.69 | * | *  | .   | 2.17  | 1.60 |
|    | Arg | 76       | A | .  | .   | .  | . | T  | .   | 1.83  | -0.69 | . | *  | F   | 2.66  | 2.16 |
| 25 | Gly | 77       | . | .  | .   | .  | T | T  | .   | 2.53  | -1.19 | . | *  | F   | 3.40  | 2.79 |
|    | Arg | 78       | . | .  | .   | .  | T | T  | .   | 2.91  | -1.57 | . | *  | F   | 3.06  | 4.02 |
|    | His | 79       | . | .  | .   | .  | . | .  | C   | 2.91  | -1.64 | . | *  | F   | 2.66  | 3.17 |
|    | Glu | 80       | . | .  | .   | .  | . | .  | C   | 2.36  | -1.26 | . | *  | F   | 2.66  | 4.29 |
|    | Arg | 81       | . | .  | .   | .  | . | T  | C   | 1.93  | -1.19 | . | *  | F   | 2.86  | 2.21 |
| 30 | Pro | 82       | . | .  | .   | .  | T | T  | .   | 1.97  | -0.70 | . | .  | F   | 3.06  | 2.35 |
|    | Ser | 83       | . | .  | .   | .  | T | T  | .   | 1.86  | -0.71 | . | *  | F   | 3.40  | 1.96 |
|    | Ala | 84       | . | .  | .   | .  | T | T  | .   | 1.22  | -0.31 | . | *  | P   | 2.76  | 1.73 |
|    | Thr | 85       | . | .  | .   | B  | T | .  | .   | 1.01  | 0.26  | . | *  | F   | 1.27  | 0.60 |
| 35 | Thr | 86       | . | .  | .   | B  | T | .  | .   | 0.04  | 0.26  | . | *  | F   | 0.93  | 0.69 |
|    | Gln | 87       | . | .  | .   | B  | T | .  | .   | -0.56 | 0.51  | . | .  | F   | 0.29  | 0.51 |
|    | Cys | 88       | . | .  | B   | B  | . | .  | .   | -0.14 | 0.70  | * | .  | .   | -0.60 | 0.29 |
|    | Pro | 89       | . | .  | B   | B  | . | .  | .   | 0.23  | 0.21  | . | *  | .   | -0.30 | 0.39 |
|    | Val | 90       | . | .  | .   | B  | . | .  | C   | 0.54  | 0.16  | . | .  | .   | -0.10 | 0.35 |
|    | Leu | 91       | . | A  | .   | .  | . | .  | C   | 0.86  | -0.24 | . | .  | .   | 0.65  | 1.14 |
| 40 | Arg | 92       | . | A  | .   | .  | . | .  | C   | 0.00  | -0.81 | * | .  | F   | 1.10  | 1.27 |
|    | Pro | 93       | A | A  | .   | .  | . | .  | .   | -0.14 | -0.60 | * | *  | F   | 0.90  | 1.27 |
|    | Glu | 94       | A | A  | .   | .  | . | .  | .   | 0.07  | -0.56 | * | *  | F   | 0.90  | 1.27 |
|    | Glu | 95       | A | A  | .   | .  | . | .  | .   | 0.33  | -1.24 | * | *  | F   | 0.90  | 1.13 |
|    | Val | 96       | A | A  | .   | .  | . | .  | .   | 1.14  | -0.74 | . | *  | .   | 0.60  | 0.74 |
| 45 | Leu | 97       | A | A  | .   | .  | . | .  | .   | 0.72  | -1.17 | * | *  | .   | 0.60  | 0.71 |
|    | Glu | 98       | A | A  | .   | .  | . | .  | .   | 0.90  | -0.69 | . | .  | .   | 0.60  | 0.59 |
|    | Ala | 99       | A | A  | .   | .  | . | .  | .   | 0.90  | -0.19 | . | *  | F   | 0.60  | 1.08 |
|    | Asp | 100      | A | .  | .   | .  | . | T  | .   | 1.01  | -0.43 | . | *  | F   | 1.00  | 2.28 |
|    | Thr | 101      | A | .  | .   | .  | . | T  | .   | 1.57  | -1.11 | * | *  | F   | 1.30  | 2.58 |
| 50 | His | 102      | A | .  | .   | .  | . | T  | .   | 1.49  | -0.73 | * | *  | F   | 1.30  | 3.42 |
|    | Gln | 103      | . | .  | .   | .  | T | T  | .   | 1.19  | -0.54 | * | .  | F   | 1.91  | 1.43 |
|    | Arg | 104      | . | .  | .   | B  | T | .  | .   | 1.57  | -0.16 | * | .  | F   | 1.42  | 1.33 |
|    | Ser | 105      | . | .  | .   | B  | T | .  | .   | 1.28  | -0.21 | * | *  | F   | 1.63  | 1.51 |
|    | Ile | 106      | . | .  | .   | B  | . | .  | C   | 1.70  | 0.20  | * | *  | F   | 0.89  | 0.92 |
| 55 | Ser | 107      | . | .  | .   | .  | . | T  | C   | 1.49  | -0.20 | * | *  | F   | 2.10  | 0.92 |
|    | Pro | 108      | . | .  | .   | .  | T | T  | .   | 1.60  | 0.56  | * | *  | F   | 1.34  | 1.07 |
|    | Trp | 109      | . | .  | .   | .  | T | T  | .   | 0.63  | 0.17  | * | *  | .   | 1.28  | 3.00 |
|    | Arg | 110      | . | .  | .   | .  | . | T  | C   | 0.93  | 0.13  | . | *  | .   | 0.87  | 1.66 |
|    | Tyr | 111      | . | .  | .   | B  | T | .  | .   | 1.51  | -0.26 | . | *  | .   | 1.40  | 1.80 |
| 60 | Arg | 112      | . | .  | .   | B  | T | .  | .   | 1.81  | -0.20 | . | *  | .   | 1.53  | 2.46 |

60099805-091098

Table I (continued)

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  | IX    | X | XI | XII | XIII  | XIV  |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|-------|---|----|-----|-------|------|
| 5  | Val | 113      | . | .  | .   | B  | . | .  | C   | 2.02  | -1.11 | . | *  | .   | 1.97  | 2.10 |
|    | Asp | 114      | . | .  | .   | .  | T | T  | .   | 2.31  | -1.11 | . | *  | F   | 3.06  | 2.32 |
|    | Thr | 115      | . | .  | .   | .  | T | T  | .   | 2.31  | -1.87 | . | *  | F   | 3.40  | 1.98 |
|    | Asp | 116      | . | .  | .   | .  | T | T  | .   | 2.31  | -1.87 | * | *  | F   | 3.06  | 5.23 |
|    | Glu | 117      | . | .  | .   | .  | T | T  | .   | 1.99  | -1.76 | * | *  | F   | 2.72  | 4.90 |
| 10 | Asp | 118      | . | .  | .   | .  | T | T  | .   | 2.84  | -1.33 | * | .  | F   | 2.38  | 5.25 |
|    | Arg | 119      | A | .  | .   | .  | . | T  | .   | 2.89  | -1.41 | * | *  | F   | 1.64  | 5.45 |
|    | Tyr | 120      | A | .  | .   | .  | . | T  | .   | 2.39  | -1.41 | * | .  | F   | 1.30  | 6.29 |
|    | Pro | 121      | A | .  | .   | .  | . | T  | .   | 1.80  | -0.73 | * | *  | F   | 1.30  | 3.11 |
|    | Gln | 122      | A | A  | .   | .  | . | .  | .   | 1.10  | -0.23 | * | *  | F   | 0.60  | 1.60 |
| 15 | Lys | 123      | A | A  | .   | .  | . | .  | .   | 0.51  | 0.56  | * | *  | F   | -0.45 | 0.89 |
|    | Leu | 124      | A | A  | .   | .  | . | .  | .   | 0.40  | 0.30  | * | .  | .   | -0.30 | 0.58 |
|    | Ala | 125      | A | A  | .   | .  | . | .  | .   | -0.02 | -0.13 | . | .  | .   | 0.30  | 0.58 |
|    | Phe | 126      | A | A  | .   | .  | . | .  | .   | -0.62 | 0.04  | . | .  | .   | -0.30 | 0.16 |
| 20 | Ala | 127      | A | A  | .   | .  | . | .  | .   | -1.29 | 0.73  | * | .  | .   | -0.60 | 0.16 |
|    | Glu | 128      | A | A  | .   | .  | . | .  | .   | -1.22 | 0.61  | * | *  | .   | -0.60 | 0.08 |
|    | Cys | 129      | A | A  | .   | .  | . | .  | .   | -0.76 | 0.11  | * | .  | .   | -0.30 | 0.19 |
|    | Leu | 130      | A | A  | .   | .  | . | .  | .   | -0.83 | -0.24 | * | *  | .   | 0.30  | 0.18 |
|    | Cys | 131      | . | .  | .   | .  | T | T  | .   | -1.02 | -0.17 | * | *  | .   | 1.10  | 0.06 |
|    | Arg | 132      | . | .  | .   | .  | T | T  | .   | -0.43 | 0.51  | * | *  | .   | 0.20  | 0.07 |
| 25 | Gly | 133      | . | .  | .   | .  | T | T  | .   | -1.02 | -0.06 | * | *  | .   | 1.10  | 0.15 |
|    | Cys | 134      | . | .  | .   | .  | T | T  | .   | -0.24 | -0.24 | * | *  | .   | 1.40  | 0.28 |
|    | Ile | 135      | A | .  | .   | .  | . | .  | .   | 0.26  | -0.81 | * | *  | .   | 1.40  | 0.28 |
|    | Asp | 136      | . | .  | .   | .  | T | .  | .   | 0.58  | -0.33 | . | *  | .   | 1.80  | 0.41 |
|    | Ala | 137      | . | .  | .   | .  | T | .  | .   | 0.58  | -0.33 | . | *  | .   | 2.10  | 0.76 |
| 30 | Arg | 138      | . | .  | .   | .  | . | T  | C   | 0.92  | -0.90 | * | *  | F   | 3.00  | 2.12 |
|    | Thr | 139      | . | .  | .   | .  | . | T  | C   | 1.28  | -1.59 | * | *  | F   | 2.70  | 2.20 |
|    | Gly | 140      | . | .  | .   | .  | . | T  | C   | 1.58  | -1.10 | * | *  | F   | 2.40  | 3.14 |
|    | Arg | 141      | A | .  | .   | .  | . | T  | .   | 0.99  | -1.10 | * | *  | F   | 1.90  | 1.62 |
|    | Glu | 142      | A | A  | .   | .  | . | .  | .   | 0.77  | -0.60 | * | *  | F   | 1.20  | 1.13 |
| 35 | Thr | 143      | A | A  | .   | .  | . | .  | .   | 0.66  | -0.40 | * | .  | F   | 0.45  | 0.94 |
|    | Ala | 144      | A | A  | .   | .  | . | .  | .   | 0.67  | -0.43 | . | *  | .   | 0.30  | 0.78 |
|    | Ala | 145      | A | A  | .   | .  | . | .  | .   | 0.16  | -0.04 | . | *  | .   | 0.30  | 0.60 |
|    | Leu | 146      | A | .  | .   | B  | . | .  | .   | 0.16  | 0.60  | . | *  | .   | -0.60 | 0.31 |
|    | Asn | 147      | A | .  | .   | B  | . | .  | .   | -0.66 | 0.11  | * | .  | .   | -0.30 | 0.60 |
| 40 | Ser | 148      | A | .  | .   | B  | . | .  | .   | -1.16 | 0.30  | * | .  | .   | -0.30 | 0.49 |
|    | Val | 149      | A | .  | .   | B  | . | .  | .   | -0.57 | 0.49  | * | .  | .   | -0.60 | 0.49 |
|    | Arg | 150      | A | .  | .   | B  | . | .  | .   | -0.28 | 0.20  | * | .  | .   | -0.30 | 0.53 |
|    | Leu | 151      | A | .  | .   | B  | . | .  | .   | -0.28 | 0.19  | * | .  | .   | -0.30 | 0.53 |
|    | Leu | 152      | A | .  | .   | B  | . | .  | .   | -1.09 | 0.49  | * | *  | .   | -0.60 | 0.58 |
| 45 | Gln | 153      | A | .  | .   | B  | . | .  | .   | -1.64 | 0.53  | * | *  | .   | -0.60 | 0.25 |
|    | Ser | 154      | A | .  | .   | B  | . | .  | .   | -1.60 | 1.17  | . | *  | .   | -0.60 | 0.22 |
|    | Leu | 155      | . | .  | B   | B  | . | .  | .   | -1.60 | 1.17  | * | .  | .   | -0.60 | 0.22 |
|    | Leu | 156      | . | .  | B   | B  | . | .  | .   | -0.68 | 0.49  | * | *  | .   | -0.60 | 0.25 |
|    | Val | 157      | . | .  | B   | B  | . | .  | .   | 0.24  | 0.09  | * | *  | .   | -0.30 | 0.37 |
| 50 | Leu | 158      | . | .  | B   | B  | . | .  | .   | 0.03  | -0.30 | * | .  | .   | 0.30  | 0.87 |
|    | Arg | 159      | . | .  | .   | B  | T | .  | .   | -0.33 | -0.56 | . | .  | F   | 1.30  | 1.63 |
|    | Arg | 160      | . | .  | .   | B  | T | .  | .   | 0.18  | -0.67 | . | *  | F   | 1.30  | 1.18 |
|    | Arg | 161      | . | .  | .   | B  | . | .  | C   | 1.10  | -0.93 | * | *  | F   | 1.10  | 1.91 |
|    | Pro | 162      | . | .  | .   | .  | T | .  | .   | 1.96  | -1.61 | . | *  | F   | 1.84  | 1.91 |
| 55 | Cys | 163      | . | .  | .   | .  | T | .  | .   | 2.42  | -1.61 | . | *  | F   | 2.18  | 1.63 |
|    | Ser | 164      | . | .  | .   | .  | T | T  | .   | 2.01  | -1.19 | . | *  | F   | 2.57  | 0.82 |
|    | Arg | 165      | . | .  | .   | .  | T | T  | .   | 1.56  | -0.80 | * | .  | F   | 2.91  | 0.71 |
|    | Asp | 166      | . | .  | .   | .  | T | T  | .   | 0.63  | -0.80 | * | *  | F   | 3.40  | 1.32 |
|    | Gly | 167      | . | .  | .   | .  | T | T  | .   | 0.63  | -0.69 | * | .  | F   | 2.91  | 0.81 |
| 60 | Ser | 168      | . | .  | .   | .  | T | .  | .   | 0.99  | -0.64 | * | .  | F   | 2.37  | 0.64 |

960150-50266009

Table I (continued)

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  | IX    | X | XI | XII | XIII  | XIV  |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|-------|---|----|-----|-------|------|
| 5  | Gly | 169      | . | .  | .   | .  | . | .  | C   | 1.08  | -0.16 | * | .  | F   | 1.53  | 0.55 |
|    | Leu | 170      | . | .  | .   | .  | . | .  | C   | 0.62  | 0.27  | * | .  | F   | 0.59  | 0.87 |
|    | Pro | 171      | . | .  | .   | .  | . | .  | C   | 0.03  | 0.27  | . | .  | F   | 0.25  | 0.64 |
|    | Thr | 172      | . | .  | .   | .  | . | T  | C   | -0.32 | 0.39  | . | .  | F   | 0.45  | 0.65 |
|    | Pro | 173      | . | .  | .   | .  | . | T  | C   | -0.61 | 0.74  | . | .  | F   | 0.15  | 0.68 |
| 10 | Gly | 174      | . | .  | .   | .  | . | T  | C   | -0.97 | 0.56  | . | .  | F   | 0.15  | 0.45 |
|    | Ala | 175      | A | .  | .   | .  | . | T  | .   | -0.19 | 0.91  | . | .  | .   | -0.20 | 0.27 |
|    | Phe | 176      | A | A  | .   | .  | . | .  | .   | -0.29 | 0.93  | . | .  | .   | -0.60 | 0.24 |
|    | Ala | 177      | A | A  | .   | .  | . | .  | .   | 0.02  | 0.99  | . | *  | .   | -0.60 | 0.34 |
|    | Phe | 178      | A | A  | .   | .  | . | .  | .   | -0.47 | 0.56  | . | .  | .   | -0.60 | 0.59 |
| 15 | His | 179      | A | A  | .   | .  | . | .  | .   | -1.01 | 0.84  | . | .  | .   | -0.60 | 0.59 |
|    | Thr | 180      | A | .  | .   | B  | . | .  | .   | -0.46 | 0.74  | . | .  | .   | -0.60 | 0.41 |
|    | Glu | 181      | A | .  | .   | B  | . | .  | .   | -0.61 | 0.74  | . | .  | .   | -0.60 | 0.64 |
|    | Phe | 182      | A | .  | .   | B  | . | .  | .   | -0.23 | 0.60  | . | .  | .   | -0.60 | 0.35 |
|    | Ile | 183      | . | .  | .   | B  | T | .  | .   | -0.39 | 0.53  | . | .  | .   | -0.20 | 0.38 |
| 20 | His | 184      | . | .  | .   | B  | T | .  | .   | -0.70 | 0.69  | . | .  | .   | -0.20 | 0.16 |
|    | Val | 185      | . | .  | .   | B  | . | .  | C   | -1.06 | 1.11  | . | .  | .   | -0.40 | 0.18 |
|    | Pro | 186      | . | .  | .   | .  | T | T  | .   | -1.37 | 0.90  | . | .  | .   | 0.20  | 0.14 |
|    | Val | 187      | . | .  | .   | .  | T | T  | .   | -1.33 | 0.70  | . | .  | .   | 0.20  | 0.15 |
| 25 | Gly | 188      | . | .  | .   | .  | T | T  | .   | -1.30 | 0.77  | . | *  | .   | 0.20  | 0.11 |
|    | Cys | 189      | . | .  | .   | .  | T | T  | .   | -2.08 | 0.77  | . | .  | .   | 0.20  | 0.05 |
|    | Thr | 190      | . | .  | B   | B  | . | .  | .   | -1.43 | 1.03  | . | *  | .   | -0.60 | 0.06 |
|    | Cys | 191      | . | .  | B   | B  | . | .  | .   | -1.11 | 0.81  | . | .  | .   | -0.60 | 0.09 |
|    | Val | 192      | . | .  | B   | B  | . | .  | .   | -0.56 | 0.39  | * | .  | .   | -0.30 | 0.33 |
| 30 | Leu | 193      | . | .  | B   | .  | . | T  | .   | -1.07 | 0.20  | * | .  | .   | 0.28  | 0.31 |
|    | Pro | 194      | . | .  | B   | .  | . | T  | .   | -0.79 | 0.36  | * | .  | F   | 0.61  | 0.42 |
|    | Arg | 195      | . | .  | .   | .  | T | T  | .   | -0.87 | 0.21  | * | .  | .   | 1.04  | 0.73 |
|    | Ser | 196      | . | .  | .   | .  | T | T  | .   | -0.59 | -0.00 | * | .  | .   | 1.97  | 1.13 |
|    | Val | 197      | . | .  | .   | .  | T | .  | .   | -0.12 | -0.26 | * | .  | .   | 1.80  | 0.93 |

860150-5086609

Table II

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  |   | IX | X | XI    | XII  | XIII |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|---|----|---|-------|------|------|
| 5  | Asn | 1        | . | .  | .   | .  | . | .  | C   | 0.58  | . | *  | . | 0.85  | 1.60 |      |
|    | Ser | 2        | . | A  | .   | .  | . | .  | C   | 1.08  | . | *  | . | 0.65  | 1.26 |      |
|    | Ala | 3        | . | A  | B   | .  | . | .  | .   | 0.88  | . | *  | . | 0.75  | 1.93 |      |
|    | Arg | 4        | . | A  | B   | .  | . | .  | .   | 0.41  | . | *  | . | 0.75  | 1.22 |      |
|    | Ala | 5        | . | A  | B   | .  | . | .  | .   | -0.01 | . | *  | . | 0.30  | 0.67 |      |
| 10 | Arg | 6        | . | A  | B   | .  | . | .  | .   | -0.31 | . | *  | . | 0.30  | 0.55 |      |
|    | Ala | 7        | . | A  | B   | .  | . | .  | .   | -0.60 | . | *  | . | 0.30  | 0.38 |      |
|    | Val | 8        | . | A  | B   | .  | . | .  | .   | -0.71 | . | *  | . | -0.30 | 0.38 |      |
|    | Leu | 9        | . | A  | B   | .  | . | .  | .   | -0.86 | * | *  | . | -0.60 | 0.17 |      |
|    | Ser | 10       | . | A  | B   | .  | . | .  | .   | -0.30 | * | *  | . | -0.60 | 0.22 |      |
| 15 | Ala | 11       | . | A  | B   | .  | . | .  | .   | -0.72 | * | .  | . | -0.60 | 0.41 |      |
|    | Phe | 12       | . | A  | B   | .  | . | .  | .   | -0.94 | * | .  | . | -0.60 | 0.72 |      |
|    | His | 13       | . | A  | B   | .  | . | .  | .   | -0.09 | * | .  | . | -0.60 | 0.44 |      |
|    | His | 14       | . | A  | B   | .  | . | .  | .   | -0.09 | * | .  | . | -0.60 | 0.76 |      |
|    | Thr | 15       | . | A  | B   | .  | . | .  | .   | -0.13 | * | .  | . | -0.60 | 0.72 |      |
| 20 | Leu | 16       | . | A  | .   | .  | . | .  | C   | 0.24  | * | *  | . | -0.10 | 0.52 |      |
|    | Gln | 17       | . | A  | .   | .  | T | .  | .   | 1.06  | * | *  | . | 0.40  | 0.60 |      |
|    | Leu | 18       | . | A  | .   | .  | . | .  | C   | 1.09  | . | *  | . | 0.80  | 0.81 |      |
|    | Gly | 19       | . | .  | .   | .  | . | T  | C   | 1.12  | . | *  | F | 2.40  | 1.70 |      |
|    | Pro | 20       | . | .  | .   | .  | . | T  | C   | 0.84  | * | *  | F | 3.00  | 1.70 |      |
| 25 | Arg | 21       | . | .  | .   | .  | . | T  | C   | 1.77  | * | *  | F | 2.70  | 2.08 |      |
|    | Glu | 22       | . | .  | B   | .  | . | T  | .   | 1.77  | * | *  | F | 2.20  | 4.12 |      |
|    | Gln | 23       | . | .  | B   | .  | . | .  | .   | 1.99  | * | *  | F | 1.70  | 4.28 |      |
|    | Ala | 24       | . | .  | .   | .  | T | .  | .   | 2.03  | * | *  | F | 1.80  | 2.21 |      |
|    | Arg | 25       | . | .  | .   | .  | T | .  | .   | 1.58  | * | *  | F | 1.50  | 1.71 |      |
| 30 | Asn | 26       | . | .  | .   | .  | T | .  | .   | 1.26  | * | *  | F | 1.05  | 0.53 |      |
|    | Ala | 27       | . | .  | .   | .  | T | .  | .   | 0.67  | * | .  | . | 0.90  | 0.81 |      |
|    | Ser | 28       | . | .  | B   | .  | . | .  | .   | 0.32  | . | .  | . | 0.78  | 0.42 |      |
|    | Cys | 29       | . | .  | B   | .  | . | T  | .   | 0.57  | . | *  | . | 0.66  | 0.26 |      |
|    | Pro | 30       | . | .  | .   | .  | T | T  | .   | 0.57  | . | *  | . | 1.34  | 0.25 |      |
| 35 | Ala | 31       | . | .  | .   | .  | T | T  | .   | 0.36  | . | *  | F | 2.37  | 0.37 |      |
|    | Gly | 32       | . | .  | .   | .  | T | T  | .   | 0.36  | . | .  | F | 2.80  | 1.06 |      |
|    | Gly | 33       | . | .  | .   | .  | . | .  | C   | 0.66  | * | *  | F | 1.97  | 0.69 |      |
|    | Arg | 34       | . | .  | B   | .  | . | .  | .   | 1.43  | * | .  | F | 1.94  | 1.15 |      |
|    | Pro | 35       | . | .  | B   | .  | . | T  | .   | 1.76  | * | .  | F | 1.86  | 2.27 |      |
| 40 | Ala | 36       | . | .  | B   | .  | . | T  | .   | 1.64  | * | *  | F | 1.58  | 4.49 |      |
|    | Asp | 37       | . | .  | B   | .  | . | T  | .   | 2.10  | * | *  | F | 1.30  | 1.99 |      |
|    | Arg | 38       | . | .  | B   | .  | . | T  | .   | 2.23  | * | *  | F | 1.30  | 2.52 |      |
|    | Arg | 39       | . | .  | B   | .  | . | .  | .   | 1.91  | * | *  | F | 1.10  | 3.85 |      |
|    | Phe | 40       | . | .  | B   | .  | . | .  | .   | 1.81  | * | *  | F | 1.44  | 3.57 |      |
| 45 | Arg | 41       | . | .  | B   | .  | . | .  | .   | 2.40  | * | *  | F | 1.78  | 2.63 |      |
|    | Pro | 42       | . | .  | .   | .  | . | T  | C   | 1.59  | . | *  | F | 2.22  | 2.16 |      |
|    | Pro | 43       | . | .  | .   | .  | T | T  | .   | 1.59  | . | *  | F | 2.16  | 2.05 |      |
|    | Thr | 44       | . | .  | .   | .  | T | T  | .   | 1.18  | . | *  | F | 3.40  | 2.05 |      |
|    | Asn | 45       | . | .  | .   | .  | . | T  | C   | 1.02  | * | *  | F | 2.56  | 1.78 |      |
| 50 | Leu | 46       | . | .  | B   | B  | . | .  | .   | 0.61  | * | *  | F | 0.87  | 0.85 |      |
|    | Arg | 47       | . | .  | B   | B  | . | .  | .   | 0.61  | * | .  | F | 1.13  | 0.79 |      |
|    | Ser | 48       | . | .  | B   | B  | . | .  | .   | 0.53  | * | .  | F | 0.79  | 0.76 |      |
|    | Val | 49       | . | .  | B   | B  | . | .  | .   | 0.26  | * | .  | F | -0.45 | 0.97 |      |
|    | Ser | 50       | . | .  | B   | .  | . | T  | .   | 0.01  | * | *  | F | 0.25  | 0.50 |      |
| 55 | Pro | 51       | . | .  | B   | .  | . | T  | .   | 0.93  | * | *  | . | -0.20 | 0.59 |      |
|    | Trp | 52       | . | .  | B   | .  | . | T  | .   | -0.07 | * | *  | . | -0.05 | 1.55 |      |
|    | Ala | 53       | . | .  | B   | .  | . | T  | .   | -0.07 | * | *  | . | -0.20 | 0.81 |      |
|    | Tyr | 54       | . | .  | B   | B  | . | .  | .   | 0.54  | * | *  | . | -0.60 | 0.70 |      |
|    | Arg | 55       | . | .  | B   | B  | . | .  | .   | 0.84  | * | *  | . | -0.45 | 1.05 |      |
| 60 | Ile | 56       | . | .  | B   | B  | . | .  | .   | 0.84  | * | *  | . | 0.13  | 1.73 |      |

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Table II (continued)

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  | IX | X | XI | XII   | XIII |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|----|---|----|-------|------|
| 5  | Ser | 57       | . | .  | B   | .  | . | .  | .   | 0.54  | *  | * | .  | 0.61  | 1.71 |
|    | Tyr | 58       | . | .  | .   | .  | T | .  | .   | 1.24  | *  | * | .  | 1.74  | 0.88 |
|    | Asp | 59       | . | .  | .   | .  | . | T  | C   | 1.24  | *  | * | F  | 2.32  | 2.46 |
|    | Pro | 60       | . | .  | .   | .  | T | T  | .   | 0.92  | *  | . | F  | 2.80  | 2.88 |
|    | Ala | 61       | . | .  | .   | .  | T | T  | .   | 1.92  | *  | . | F  | 2.52  | 2.84 |
| 10 | Arg | 62       | . | .  | B   | .  | . | T  | .   | 1.98  | *  | . | F  | 2.14  | 3.33 |
|    | Tyr | 63       | . | .  | B   | .  | . | T  | .   | 1.41  | *  | . | .  | 1.41  | 3.37 |
|    | Pro | 64       | . | .  | B   | .  | . | T  | .   | 1.20  | *  | . | .  | 0.53  | 2.75 |
|    | Arg | 65       | . | .  | .   | .  | T | T  | .   | 1.41  | *  | . | .  | 0.65  | 2.17 |
|    | Tyr | 66       | . | .  | B   | .  | . | T  | .   | 1.41  | *  | . | F  | 0.40  | 2.40 |
| 15 | Leu | 67       | . | .  | B   | .  | . | .  | .   | 1.06  | *  | . | F  | 0.80  | 1.57 |
|    | Pro | 68       | . | .  | B   | .  | . | .  | .   | 0.63  | *  | . | .  | 0.05  | 1.26 |
|    | Glu | 69       | . | .  | .   | .  | T | .  | .   | 0.03  | *  | . | .  | 0.00  | 0.43 |
|    | Ala | 70       | . | .  | B   | B  | . | .  | .   | -0.74 | *  | . | .  | -0.60 | 0.43 |
|    | Tyr | 71       | . | .  | B   | B  | . | .  | .   | -0.39 | *  | . | .  | -0.60 | 0.15 |
| 20 | Cys | 72       | . | .  | B   | B  | . | .  | .   | 0.08  | *  | . | .  | -0.30 | 0.17 |
|    | Leu | 73       | . | .  | B   | B  | . | .  | .   | -0.38 | .  | * | .  | -0.60 | 0.16 |
|    | Cys | 74       | . | .  | B   | .  | . | T  | .   | -1.19 | .  | * | .  | -0.20 | 0.06 |
|    | Arg | 75       | . | .  | B   | .  | . | T  | .   | -0.91 | *  | * | .  | -0.20 | 0.09 |
|    | Gly | 76       | . | .  | B   | .  | . | T  | .   | -1.01 | *  | . | .  | -0.20 | 0.15 |
| 25 | Cys | 77       | . | .  | B   | .  | . | T  | .   | -1.16 | .  | * | .  | 0.10  | 0.28 |
|    | Leu | 78       | . | .  | B   | B  | . | .  | .   | -1.04 | .  | . | .  | -0.30 | 0.12 |
|    | Thr | 79       | . | .  | B   | B  | . | .  | .   | -0.72 | .  | * | .  | -0.60 | 0.10 |
|    | Gly | 80       | . | .  | .   | B  | . | .  | C   | -0.83 | .  | * | .  | -0.40 | 0.19 |
|    | Leu | 81       | . | .  | .   | B  | . | .  | C   | -0.49 | .  | . | .  | -0.40 | 0.40 |
| 30 | Phe | 82       | . | .  | B   | B  | . | .  | .   | 0.18  | .  | . | F  | 0.45  | 0.48 |
|    | Gly | 83       | . | .  | .   | B  | . | .  | C   | 0.13  | .  | * | F  | 0.95  | 0.81 |
|    | Glu | 84       | . | A  | B   | .  | . | .  | .   | 0.56  | .  | * | F  | 0.45  | 0.73 |
|    | Glu | 85       | . | A  | B   | .  | . | .  | .   | 0.20  | .  | * | F  | 0.90  | 1.65 |
|    | Asp | 86       | . | A  | B   | B  | . | .  | .   | 1.12  | .  | * | F  | 0.90  | 1.45 |
| 35 | Val | 87       | . | A  | B   | B  | . | .  | .   | 1.52  | .  | * | F  | 0.90  | 1.63 |
|    | Arg | 88       | . | A  | .   | B  | T | .  | .   | 1.28  | .  | * | .  | 1.15  | 1.26 |
|    | Phe | 89       | . | A  | .   | B  | T | .  | .   | 1.07  | .  | * | .  | 1.00  | 0.77 |
|    | Arg | 90       | . | A  | .   | B  | T | .  | .   | 0.21  | .  | * | .  | 0.85  | 1.59 |
|    | Ser | 91       | . | A  | .   | B  | . | .  | C   | -0.03 | .  | * | .  | 0.50  | 0.60 |
| 40 | Ala | 92       | . | .  | .   | B  | . | .  | C   | 0.22  | .  | * | .  | -0.25 | 1.09 |
|    | Pro | 93       | . | .  | .   | B  | . | .  | C   | -0.10 | .  | * | .  | -0.10 | 0.55 |
|    | Val | 94       | . | .  | .   | B  | T | .  | .   | 0.29  | .  | * | .  | -0.20 | 0.64 |
|    | Tyr | 95       | . | .  | B   | B  | . | .  | .   | -0.68 | *  | . | .  | -0.60 | 0.91 |
|    | Met | 96       | . | .  | B   | B  | . | .  | .   | -1.23 | .  | . | .  | -0.60 | 0.44 |
| 45 | Pro | 97       | . | .  | B   | B  | . | .  | .   | -1.46 | .  | * | .  | -0.60 | 0.44 |
|    | Thr | 98       | . | .  | B   | B  | . | .  | .   | -1.13 | *  | . | .  | -0.60 | 0.23 |
|    | Val | 99       | . | .  | B   | B  | . | .  | .   | -0.17 | *  | . | .  | -0.60 | 0.46 |
|    | Val | 100      | . | .  | B   | B  | . | .  | .   | -0.23 | .  | . | .  | 0.30  | 0.58 |
|    | Leu | 101      | . | .  | B   | B  | . | .  | .   | 0.16  | .  | . | .  | 0.30  | 0.58 |
| 50 | Arg | 102      | . | .  | B   | B  | . | .  | .   | -0.22 | .  | . | F  | 0.60  | 1.20 |
|    | Arg | 103      | . | .  | B   | B  | . | .  | .   | -0.58 | .  | . | F  | 0.60  | 1.63 |
|    | Thr | 104      | . | .  | B   | B  | . | .  | .   | -0.31 | .  | . | F  | 0.60  | 1.06 |
|    | Pro | 105      | . | .  | B   | B  | . | .  | .   | 0.20  | *  | . | F  | 1.00  | 0.55 |
|    | Ala | 106      | . | .  | B   | .  | . | .  | .   | 0.67  | .  | * | .  | 1.00  | 0.28 |
| 55 | Cys | 107      | . | .  | B   | .  | . | T  | .   | 0.67  | .  | . | .  | 0.85  | 0.19 |
|    | Ala | 108      | . | .  | .   | .  | T | T  | .   | 0.26  | *  | * | .  | 2.10  | 0.24 |
|    | Gly | 109      | . | .  | .   | .  | T | T  | .   | -0.29 | *  | . | F  | 2.50  | 0.32 |
|    | Gly | 110      | . | .  | .   | .  | T | T  | .   | -0.32 | *  | . | F  | 2.25  | 0.44 |
|    | Arg | 111      | . | .  | B   | B  | . | .  | .   | -0.04 | *  | . | F  | 0.60  | 0.69 |
| 60 | Ser | 112      | . | .  | B   | B  | . | .  | .   | 0.62  | *  | . | F  | 0.35  | 1.00 |

860160-5086008

Table II (continued)

|    | Res | Position | I | II | III | IV | V | VI | VII | VIII  |   | IX | X | XI | XII   | XIII |
|----|-----|----------|---|----|-----|----|---|----|-----|-------|---|----|---|----|-------|------|
| 5  | Val | 113      | . | .  | B   | B  | . | .  | .   | 0.62  | * | .  | . | .  | 0.70  | 1.75 |
|    | Tyr | 114      | . | .  | B   | .  | . | .  | .   | 0.72  | . | .  | . | .  | 0.50  | 0.90 |
|    | Thr | 115      | . | .  | B   | .  | . | .  | .   | 0.21  | . | .  | . | .  | -0.25 | 1.05 |
|    | Glu | 116      | . | .  | B   | B  | . | .  | .   | -0.21 | . | *  | . | .  | -0.45 | 1.05 |
|    | Ala | 117      | . | .  | B   | B  | . | .  | .   | -0.80 | . | *  | . | .  | -0.60 | 0.97 |
| 10 | Tyr | 118      | . | .  | B   | B  | . | .  | .   | -0.16 | . | *  | . | .  | -0.60 | 0.47 |
|    | Val | 119      | . | .  | B   | B  | . | .  | .   | -0.77 | . | *  | . | .  | -0.60 | 0.42 |
|    | Thr | 120      | . | .  | B   | B  | . | .  | .   | -0.80 | . | *  | . | .  | -0.60 | 0.31 |
|    | Ile | 121      | . | .  | B   | B  | . | .  | .   | -1.47 | . | *  | . | .  | -0.60 | 0.20 |
|    | Pro | 122      | . | .  | B   | .  | . | T  | .   | -1.19 | . | *  | . | .  | -0.20 | 0.14 |
| 15 | Val | 123      | . | .  | .   | .  | T | T  | .   | -1.61 | . | .  | . | .  | 0.20  | 0.14 |
|    | Gly | 124      | . | .  | .   | .  | T | T  | .   | -1.61 | . | .  | . | .  | 0.20  | 0.11 |
|    | Cys | 125      | . | .  | B   | .  | . | T  | .   | -1.51 | . | .  | . | .  | -0.20 | 0.05 |
|    | Thr | 126      | . | .  | B   | .  | . | .  | .   | -0.62 | . | .  | . | .  | -0.40 | 0.11 |
|    | Cys | 127      | . | .  | B   | .  | . | .  | .   | -0.62 | . | .  | . | .  | -0.10 | 0.19 |
| 20 | Val | 128      | . | .  | B   | .  | . | T  | .   | 0.23  | . | .  | . | .  | 0.40  | 0.55 |
|    | Pro | 129      | . | .  | B   | .  | . | T  | .   | 0.62  | . | .  | . | F  | 1.45  | 0.65 |
|    | Glu | 130      | . | .  | B   | .  | . | T  | .   | 1.29  | * | .  | . | F  | 2.20  | 2.44 |
|    | Pro | 131      | . | .  | B   | .  | . | T  | .   | 1.01  | * | .  | . | F  | 2.50  | 5.49 |
|    | Glu | 132      | . | .  | .   | .  | T | .  | .   | 1.68  | * | .  | . | F  | 3.00  | 3.59 |
| 25 | Lys | 133      | A | .  | .   | .  | . | .  | .   | 2.23  | * | .  | . | F  | 2.30  | 3.46 |
|    | Asp | 134      | A | .  | .   | .  | . | T  | .   | 1.56  | * | .  | . | F  | 2.20  | 3.00 |
|    | Ala | 135      | A | .  | .   | .  | . | T  | .   | 1.56  | * | .  | . | F  | 1.90  | 1.21 |
|    | Asp | 136      | A | .  | .   | .  | . | T  | .   | 1.47  | * | .  | . | F  | 1.45  | 0.98 |
|    | Ser | 137      | . | .  | B   | .  | . | T  | .   | 1.17  | * | .  | . | F  | 1.15  | 0.78 |
| 30 | Ile | 138      | . | .  | B   | .  | . | .  | .   | 0.23  | * | .  | . | F  | 0.80  | 1.04 |
|    | Asn | 139      | . | .  | B   | .  | . | T  | .   | 0.23  | * | .  | . | F  | 0.85  | 0.44 |
|    | Ser | 140      | . | .  | B   | .  | . | T  | .   | 0.87  | * | .  | . | F  | 1.16  | 0.54 |
|    | Ser | 141      | . | .  | B   | .  | . | T  | .   | 0.87  | * | .  | . | F  | 1.62  | 1.55 |
|    | Ile | 142      | . | .  | B   | .  | . | T  | .   | 0.82  | . | *  | . | F  | 2.23  | 1.67 |
| 35 | Asp | 143      | . | .  | B   | .  | . | T  | .   | 1.12  | * | *  | . | F  | 2.54  | 1.23 |
|    | Lys | 144      | . | .  | .   | .  | T | T  | .   | 1.17  | * | .  | . | F  | 3.10  | 0.93 |
|    | Gln | 145      | . | .  | B   | .  | . | T  | .   | 0.66  | * | .  | . | F  | 2.54  | 2.65 |
|    | Gly | 146      | . | .  | B   | .  | . | T  | .   | 0.14  | * | .  | . | F  | 2.23  | 1.31 |
|    | Ala | 147      | . | A  | B   | .  | . | .  | .   | 0.22  | * | .  | . | F  | 1.07  | 0.54 |
| 40 | Lys | 148      | . | A  | B   | .  | . | .  | .   | -0.12 | . | .  | . | F  | 0.16  | 0.26 |
|    | Leu | 149      | . | A  | B   | .  | . | .  | .   | -0.38 | * | .  | . | .  | -0.60 | 0.26 |
|    | Leu | 150      | . | A  | B   | .  | . | .  | .   | -0.38 | . | .  | . | .  | -0.60 | 0.39 |
|    | Leu | 151      | . | A  | B   | .  | . | .  | .   | -0.03 | . | .  | . | .  | -0.06 | 0.32 |
|    | Gly | 152      | . | .  | B   | .  | . | T  | .   | -0.03 | . | .  | . | F  | 0.73  | 0.64 |
| 45 | Pro | 153      | . | .  | .   | .  | . | T  | C   | -0.29 | . | .  | . | F  | 1.17  | 0.78 |
|    | Asn | 154      | . | .  | .   | .  | T | T  | .   | -0.07 | . | .  | . | F  | 2.36  | 1.47 |
|    | Asp | 155      | . | .  | .   | .  | . | T  | C   | 0.40  | . | .  | . | F  | 2.40  | 1.50 |
|    | Ala | 156      | . | .  | .   | .  | . | .  | C   | 1.00  | . | .  | . | F  | 1.81  | 0.96 |
|    | Pro | 157      | . | .  | .   | .  | . | T  | C   | 0.96  | . | .  | . | F  | 1.77  | 0.92 |
| 50 | Ala | 158      | . | .  | .   | .  | . | T  | C   | 0.78  | . | .  | . | .  | 1.38  | 0.71 |
|    | Gly | 159      | . | .  | .   | .  | . | T  | C   | 0.39  | . | .  | . | .  | 0.54  | 0.90 |
|    | Pro | 160      | . | .  | B   | .  | . | T  | .   | 0.00  | . | .  | . | .  | 0.10  | 0.74 |

860160.5086608

Among highly preferred fragments in this regard are those that comprise regions of IL-21 or IL-22 that combine several structural features, such as several of the features set out above.

- Other preferred fragments are biologically active IL-21 and IL-22 fragments.
- 5 Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the IL-21 and IL-22 polypeptides. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

#### 10 Epitopes & Antibodies

- In the present invention, "epitopes" refer to IL-21 and IL-22 polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to an IL-21 or IL-22 polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this
- 15 fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope". In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response (see, for instance, Geysen, *et al.*, *Proc. Natl. Acad. Sci. USA* **81**:3998- 4002 (1983)).

- Fragments which function as epitopes may be produced by any conventional
- 20 means (see, e.g., Houghten, R. A., *Proc. Natl. Acad. Sci. USA* **82**:5131-5135 (1985); further described in U.S. Patent No. 4,631,211).

- In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including
- 25 monoclonal antibodies, that specifically bind the epitope (see, for instance, Wilson, *et al.*, *Cell* **37**:767-778 (1984); Sutcliffe, J. G. *et al.*, *Science* **219**:660-666 (1983)).

- Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (see, for instance, Sutcliffe, *et al.*, *supra*; Wilson, *et al.*, *supra*; Chow, M., *et al.*, *Proc. Natl. Acad. Sci. USA* **82**:910-914; and Bittle, F. J., *et al.*, *J. Gen. Virol.* **66**:2347-2354 (1985)). A preferred immunogenic epitope includes
- 30 the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be
- 35 sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Using DNASTar analysis, SEQ ID NO:2 was found antigenic at amino acids: from about Arg-2 to about Pro-11, from about Cys-24 to about Glu-32, and from about Arg-51 to about Gly-59. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HTGED19. Again using DNASTar analysis, SEQ ID NO:4 was found antigenic at amino acids: from about Gly-19 to about Ala-27, from about Pro-30 to about Arg-38, from about Phe-40 to about Ser-48, from about Tyr-58 to about Leu-67, from about Pro-105 to about Val-113, from about Pro-129 to about Ser-137, from about Asn-139 to about Ala-147, and from about Leu-151 to about Gly-159. Thus, these regions can be used as epitopes to produce antibodies against the protein encoded by HFPBX96.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')<sub>2</sub> fragments) which are capable of specifically binding to protein. Fab and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl, *et al.*, *J. Nucl. Med.* 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

### Fusion Proteins

Any IL-21 or IL-22 polypeptide can be used to generate fusion proteins. For example, the IL-21 or IL-22 polypeptides, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the IL-21 or IL-22 polypeptides can be used to indirectly detect a second protein by binding to IL-21 or IL-22, respectively. Moreover, because secreted proteins target cellular locations based on trafficking signals, the IL-21 and IL-22 polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to the IL-21 and IL-22 polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the IL-21 and IL-22 polypeptides. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the IL-21 and IL-22 polypeptides to improve stability and persistence during purification from the host cell or during subsequent handling and storage. Also, peptide moieties may be



added to the IL-21 and IL-22 polypeptides to facilitate purification. Such regions may be removed prior to final preparation of the IL-21 and IL-22 polypeptides. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, IL-21 and IL-22 polypeptides, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker, *et al.*, *Nature* 331:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (Fountoulakis, *et al.*, *J. Biochem.* 270:3958-3964 (1995)).

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A 0232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (see, Bennett, D., *et al.*, *J. Mol. Recog.* 8:52-58 (1995); Johanson, K., *et al.*, *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the IL-21 and IL-22 polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of IL-21 and IL-22, respectively. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described by Gentz and coworkers (*Proc. Natl. Acad. Sci. USA* 86:821-824 (1989)), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, *et al.*, *Cell* 37:767 (1984)).

Thus, any of the above fusion proteins can be engineered using the IL-21 and IL-22 polynucleotides or the polypeptides.

#### Vectors, Host Cells, and Protein Production

5 The present invention also relates to vectors containing the IL-21 and IL-22 polynucleotides, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

10 IL-21 and IL-22 polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

15 The IL-21 and IL-22 polynucleotide inserts should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

20 As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; 25 insect cells such as *Drosophila* S2 and *Spodoptera* SF9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

30 Among vectors preferred for use in bacteria include pHE4-5 and other pHE-like vectors; pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are

pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

5 Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals (for example, Davis, *et al.*, *Basic Methods In Molecular Biology* (1986)). It is specifically contemplated that IL-21 and IL-22 polypeptides may, in fact, be expressed by a host cell lacking a recombinant  
10 vector.

IL-21 and IL-22 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity  
15 chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

IL-21 and IL-22 polypeptides, and preferably the secreted forms thereof, can also be recovered from: products purified from natural sources, including bodily  
20 fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the IL-21 and IL-22 polypeptides may be glycosylated or may be  
25 non-glycosylated. In addition, IL-21 and IL-22 polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most  
30 proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

#### Uses of the IL-21 and IL-22 Polynucleotides

35 The IL-21 and IL-22 polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Clone HTGED19 and clone HFPBX96 can each be mapped to a specific chromosome. Thus, IL-21 and IL-22 polynucleotides can then be used in linkage analysis as a marker for those specific chromosome.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1 and SEQ ID NO:3. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human IL-21 or IL-22 genes corresponding to SEQ ID NO:1 or SEQ ID NO:3, respectively, will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the IL-21 and IL-22 polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the IL-21 and IL-22 polynucleotides can also be achieved using fluorescence *in situ* hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred (For review, see Verma, *et al.*, "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988)).

For chromosome mapping, the IL-21 and IL-22 polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

In a preferred embodiment, the gene encoding IL-22 of the present invention has been mapped using FISH technology to a location on human chromosome 13 at position 13q11.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a

particular disease (disease mapping data are found, for example, in McKusick, V., Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the IL-21 and IL-22 polynucleotides and the corresponding genes between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the IL-21 and IL-22 polypeptides and the corresponding genes from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using IL-21 and IL-22 polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, an IL-21 or IL-22 polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee, *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney, *et al.*, *Science* 241:456 (1988); and Dervan, *et al.*, *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. *Neurochem.* 56:560 (1991); Oligodecoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

IL-21 and IL-22 polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. IL-21 and IL-22 offer means of targeting such

genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The IL-21 and IL-22 polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The IL-21 and IL-22 polynucleotides can be used as additional DNA markers for RFLP.

The IL-21 and IL-22 polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals (Erlich, H., *PCR Technology*, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, IL-21 and IL-22 polynucleotides can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from IL-21 and IL-22 sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

Because IL-21 is found expressed almost exclusively in apoptotic T-cells, IL-21 polynucleotides are useful as hybridization probes for differential identification of the

tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-21 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-21 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-21 gene expression level, i.e., the IL-21 expression level in healthy tissue from an individual not having the Immune system disorder.

Likewise, since IL-22 is found expressed in bone marrow, skeletal muscle, and brain, IL-22 polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to IL-22 polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of disorders of the above tissues or cells, particularly of the Immune system, significantly higher or lower levels of IL-22 gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" IL-22 gene expression level, i.e., the IL-22 expression level in healthy tissue from an individual not having the Immune system disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves:

- (a) assaying IL-21 or IL-22 gene expression level in cells or body fluid of an individual;
- (b) comparing the IL-21 or IL-22 gene expression level with a standard IL-21 or IL-22 gene expression level, respectively, whereby an increase or decrease in the assayed IL-21 or IL-22 gene expression level compared to the standard expression level is indicative of disorder in the Immune system.

In the very least, the IL-21 and IL-22 polynucleotides can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### **Uses of IL-21 and IL-22 Polypeptides**

IL-21 and IL-22 polypeptides can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

IL-21 and IL-22 polypeptides can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., *et al.*, *J. Cell. Biol.* 101:976-985 (1985); Jalkanen, M., *et al.*, *J. Cell. Biol.* 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described by Burchiel and colleagues ("Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of IL-21 or IL-22 polypeptides in cells or body fluid of an individual; (b) comparing the level of IL-21 or IL-22 gene expression with a standard gene expression level, whereby an increase or decrease in the assayed IL-21 or IL-22



polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, IL-21 and IL-22 polypeptides can be used to treat disease. For example, patients can be administered IL-21 and IL-22 polypeptides in an effort to replace absent or decreased levels of the IL-21 and IL-22 polypeptides, respectively, (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to IL-21 and IL-22 polypeptides can also be used to treat disease. For example, administration of an antibody directed to an IL-21 or IL-22 polypeptide can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the IL-21 and IL-22 polypeptides can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. IL-21 and IL-22 polypeptides can also be used to raise antibodies, which, in turn, are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, IL-21 and IL-22 polypeptides can be used to test the following biological activities.

#### **Biological Activities of IL-21 and IL-22**

IL-21 and IL-22 polynucleotides and polypeptides can be used in assays to test for one or more biological activities. If IL-21 and IL-22 polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that IL-21 and IL-22 may be involved in the diseases associated with the biological activity. Therefore, IL-21 and IL-22 could be used to treat the associated disease.

The IL-21 and IL-22 proteins of the present invention modulate IL-6 secretion from NIH-3T3 cells. An *in vitro* ELISA assay which quantitates the amount of IL-6 secreted from cells in response to treatment with cytokines or the soluble extracellular domains of cytokine receptors has been described (Yao, Z., *et al.*, *Immunity* 3:811-821 (1995)). Briefly, the assay involves plating the target cells at a density of approximately  $5 \times 10^6$  cells/mL in a volume of 500  $\mu$ L in the wells of a 24 well flat-bottomed culture plate (Costar). The cultures are then treated with various concentrations of the cytokine or the soluble extracellular domain of cytokine receptor in

question. The cells are then cultured for 24 hours at 37°C. At this time, 50 µL of supernatant is removed and assayed for the quantity of IL-6 essentially as described by the manufacturer (Genzyme, Boston, MA). IL-6 levels are then calculated by reference to a standard curve constructed with recombinant IL-17 cytokine. Such activity is useful for determining the level of IL-21- or IL-22-mediated IL-6 secretion.

IL-21 and IL-22 protein modulates immune system cell proliferation and differentiation in a dose-dependent manner in the above-described assay. Thus, "a polypeptide having IL-21 or IL-22 protein activity" includes polypeptides that also exhibit any of the same stimulatory activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the IL-21 or IL-22 proteins, preferably, "a polypeptide having IL-21 or IL-22 protein activity" will exhibit substantially similar dose-dependence in a given activity as compared to the IL-21 or IL-22 protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity relative to the reference IL-21 or IL-22 protein).

Lymphocyte proliferation is another *in vitro* assay which may be performed to determine the activity of IL-21 and IL-22. For example, Yao and colleagues (*Immunity* 3:811-821 (1995)) have recently described an *in vitro* assay for determining the effects of various cytokines and soluble cytokine receptors on the proliferation of murine leukocytes. Briefly, lymphoid organs are harvested aseptically, lymphocytes are isolated from the harvested organs, and the resulting collection of lymphoid cells are suspended in standard culture medium as described by Fanslow and coworkers (*J. Immunol.* 147:535-5540 (1991)). The lymphoid cell suspensions may then be divided into several different subclasses of lymphoid cells including splenic T-cells, lymph node B-cells, CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, and mature adult thymocytes. For splenic T-cells, spleen cell suspensions (200 x 10<sup>6</sup> cells) are incubated with CD11b mAb and class II MHC mAb for 30 min at 4°C, loaded on a T-cell purification column (Pierce, Rockford, IL), and the T-cells eluted according to the manufacturer's instructions. Using this method, purity of the resulting T-cell populations should be >95% CD3<sup>+</sup> and <1% sIgM<sup>+</sup>. For purification of lymph node subsets, B-cells are removed from by adherence to tissue culture dishes previously coated with goat anti-mouse IgG (10 µg/mL). Remaining cells were then incubated with anti-CD4 or anti-CD8 for 30 min at 4°C then washed and placed on tissue culture dishes previously coated with goat anti-rat IgG (20 µg/mL). After 45 min, nonadherent cells are removed and tested for purity by flow cytometry. CD4 and surface Ig-depleted cells should be >90% TCR-ab, CD8<sup>+</sup>, whereas CD8 and surface Ig-depleted cells should be >95% TCR-ab, CD4<sup>+</sup>. Finally, to enrich for mature adult thymocytes, cells are suspended at 10<sup>8</sup>/mL in 10%

anti-HSA and 10% low tox rabbit complement (Cedarlane, Ontario, Canada), incubated for 45 min at 37°C, and remaining viable cells isolated over Ficoll-Hypaque (Pharmacia, Piscataway, NJ). This procedure should yield between 90 and 95% CD3<sup>hi</sup> cells that are either CD4<sup>+</sup>8<sup>-</sup> or CD4<sup>+</sup>8<sup>+</sup>.

5

#### Immune Activity

IL-21 and IL-22 polypeptides or polynucleotides may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, IL-21 and IL-22 polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

IL-21 and IL-22 polynucleotides or polypeptides may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. IL-21 and IL-22 polypeptides or polynucleotides could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, IL-21 and IL-22 polypeptides or polynucleotides can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, IL-21 and IL-22 polynucleotides or polypeptides could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, IL-21 and IL-22 polynucleotides or polypeptides that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting, important in the treatment of heart attacks (infarction), strokes, or scarring.

IL-21 and IL-22 polynucleotides or polypeptides may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of IL-21 and IL-22 polypeptides or polynucleotides that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by IL-21 and IL-22 include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by IL-21 and IL-22 polypeptides or polynucleotides. Moreover, IL-21 and IL-22 can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

IL-21 and IL-22 polynucleotides or polypeptides may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of IL-21 and IL-22 polypeptides or polynucleotides that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate inflammation. For example, IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury,

inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

### **Hyperproliferative Disorders**

5 IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect hyperproliferative disorders, including neoplasms. IL-21 and IL-22 polypeptides or polynucleotides may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, IL-21 and IL-22 polypeptides or polynucleotides may proliferate other cells which can inhibit the hyperproliferative disorder.

10 For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may  
15 also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum,  
20 endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of such hyperproliferative  
25 disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

### **Infectious Disease**

IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases  
35 may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, IL-21 and

IL-22 polypeptides or polynucleotides may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

- Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

- Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by IL-21 and IL-22 polynucleotides or polypeptides include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal), Pasteurellaceae Infections (e.g., Actinobacillus, Haemophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme

Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by IL-21 polynucleotides or polypeptides include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. IL-21 and IL-22 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using IL-21 and IL-22 polypeptides or polynucleotides could either be by administering an effective amount of IL-21 or IL-22 polypeptide to the patient, or by removing cells from the patient, supplying the cells with IL-21 and IL-22 polynucleotide, and returning the engineered cells to the patient (*ex vivo* therapy). Moreover, the IL-21 and IL-22 polypeptide or polynucleotide can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### **Regeneration**

IL-21 and IL-22 polynucleotides or polypeptides can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (see, *Science* 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, IL-21 and IL-22 polynucleotides or polypeptides may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. IL-21 and IL-22 polynucleotides or polypeptides of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using IL-21 and IL-22 polynucleotides or polypeptides to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the IL-21 and IL-22 polynucleotides or polypeptides.

#### Chemotaxis

IL-21 and IL-22 polynucleotides or polypeptides may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

IL-21 and IL-22 polynucleotides or polypeptides may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, IL-21 and IL-22 could also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that IL-21 and IL-22 polynucleotides or polypeptides may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, IL-21 and IL-22 polynucleotides or polypeptides could be used as an inhibitor of chemotaxis.



### Binding Activity

IL-21 and IL-22 polypeptides may be used to screen for molecules that bind to IL-21 or IL-22 or for molecules to which IL-21 or IL-22 bind. The binding of IL-21 and IL-22 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the IL-21 and IL-22 or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of IL-21 or IL-22, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (see, Coligan, *et al.*, Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which IL-21 and IL-22 bind, or at least, a fragment of the receptor capable of being bound by IL-21 or IL-22 (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express IL-21 and IL-22, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing IL-21 and IL-22 (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either IL-21 and IL-22 or the molecule.

The assay may simply test binding of a candidate compound to IL-21 or IL-22, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to IL-21 or IL-22.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing IL-21 or IL-22, measuring IL-21/molecule or IL-22/molecule activity or binding, respectively, and comparing the IL-21/molecule or IL-22/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure IL-21 and IL-22 levels or activities in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure IL-21 and IL-22 levels or activities by either binding, directly or indirectly, to IL-21 or IL-22 or by competing with IL-21 or IL-22 for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a

particular result in a patient (e.g., blood vessel growth) by activating or inhibiting IL-21 or IL-22. Moreover, the assays can discover agents which may inhibit or enhance the production of IL-21 and IL-22 from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which  
5 bind to IL-21 and IL-22 comprising the steps of: (a) incubating a candidate binding compound with IL-21 or IL-22; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with IL-21 or IL-22, (b)  
10 assaying a biological activity, and (b) determining if a biological activity of IL-21 or IL-22, respectively, has been altered.

#### Other Activities

IL-21 and IL-22 polypeptides or polynucleotides may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above,  
15 hematopoietic lineage.

IL-21 and IL-22 polypeptides or polynucleotides may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, IL-21 and IL-22 polypeptides or polynucleotides may be used to modulate  
20 mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

IL-21 and IL-22 polypeptides or polynucleotides may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain,  
25 reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

IL-21 and IL-22 polypeptides or polynucleotides may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional  
30 components.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

#### Examples

In the case where the full-length IL-21 and the partial IL-22 are not specifically mentioned, specific details are provided in the following examples only for the partial-length IL-21 molecules of the present invention. However, the examples can also be easily performed for the full-length IL-21 and the full-length or partial-length IL-22 molecules of the present invention by using the details provided for the partial IL-21 and substituting appropriate nucleotides or amino acid residues of the full-length IL-21, the full-length or partial-length IL-22, and/or any deletion mutations or other variants of either IL-21 or IL-22, for example, in the design of suitable PCR primers, and the like. The use or applicability of another IL-21 or IL-22 in place of the IL-21 exemplified below is thus contemplated in each of the following examples. When provided with the nucleotide and amino acid sequences of IL-21 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:28, and SEQ ID NO:29) and IL-22 (SEQ ID NO:3 and SEQ ID NO:4) of the present invention, one of ordinary skill in the art could easily perform the following examples with the intent of isolating or further characterizing or manipulating another IL-21 or IL-22 in place of the IL-21 shown in the Examples below.

#### **Example 1: Isolation of the IL-21 and IL-22 cDNA Clones From the Deposited Samples**

The cDNAs encoding the partial IL-21 and IL-22 molecules are each inserted into the *Eco* RI and *Xho* I restriction sites of the multiple cloning site of pBluescript. pBluescript contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies (see, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993)).

Two approaches can be used to isolate IL-21 from the deposited sample. First, a specific polynucleotide of SEQ ID NO:1 with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide kinase and purified according to routine methods (e.g., Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982)). The plasmid mixture is transformed into a suitable host (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g.,

Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:1 (i.e., within the region of SEQ ID NO:1 bounded by the 5' and 3' nucleotides of the clone) are synthesized and used to amplify the IL-21 cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25  $\mu$ l of reaction mixture with 0.5  $\mu$ g of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $MgCl_2$ , 0.01% (w/v) gelatin, 20  $\mu$ M each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of the IL-21 gene which may not be present in the deposited clone. These methods include, but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' RACE protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript (Fromont-Racine, *et al.*, *Nucl. Acids Res.* 21(7):1683-1684 (1993)).

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the IL-21 gene of interest is used to PCR amplify the 5' portion of the IL-21 full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with a phosphatase, if necessary, to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNA. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the IL-21 gene.

#### **Example 2: Isolation of IL-21 Genomic Clones**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:1., according to the method described in Example 1 (see also, Sambrook, *et al.*, *supra*).

#### **Example 3: Tissue Distribution of IL-21**

Tissue distribution of mRNA expression of IL-21 is determined using protocols for Northern blot analysis, described by, among others, Sambrook and colleagues (*supra*). For example, an IL-21 probe produced by the method described in Example 1 is labeled with <sup>32</sup>P using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using a CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system (IM) tissues (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and the films developed according to standard procedures.

Using essentially the above-prescribed protocol, Northern blot analyses were performed to determine the expression pattern of IL-21 and IL-22. In the case of IL-21, a major message of approximately 5 kb was detected predominantly in thymus, but was also detectable in adrenal cortex, spleen, pancreas, and very weakly in lymph node, PBL, fetal liver, adrenal medulla, thyroid, small intestine, stomach, and heart. In the case of IL-22, a major message of slightly less than 1 kb was detected in

conjunction with a minor band of approximately 5 kb predominantly in testis and spinal cord, but was also detected in bone marrow and small intestine.

#### 5 Example 4: Chromosomal Mapping of IL-21

10 An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:1. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is 15 determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### 20 Example 5: Bacterial Expression of IL-21

An IL-21 polynucleotide encoding an IL-21 polypeptide of the invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, 25 such as *Bam* HI and *Hin* dIII, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, *Bam* HI and *Hin* dIII correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (*Amp*<sup>R</sup>), a bacterial origin of replication (*ori*), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme 30 cloning sites.

Specifically, to clone the mature domain of the IL-21 protein in a bacterial vector, the 5' primer has the sequence 5'-GAT CGC GGA TCC GAC ACG GAT GAG GAC CGC TAT CCA CAG AAG CTG-3' (SEQ ID NO:9) containing the underlined 35 *Bam* HI restriction site followed several nucleotides of the amino terminal coding sequence of the mature IL-21 sequence in SEQ ID NO:1. One of ordinary skill in the art would appreciate, of course, that the point in the protein coding sequence where the

5' primer begins may be varied to amplify a DNA segment encoding any desired portion of the complete IL-21 protein shorter or longer than the mature form of the protein. The 3' primer has the sequence 5'-CCC AAG CTT TCA CAC TGA ACG GGG CAG CAC GCA GGT GCA GC-3' (SEQ ID NO:10) containing the underlined *Hin* dIII restriction site followed by a number nucleotides complementary to the 3' end of the coding sequence of the IL-21 DNA sequence of SEQ ID NO:1.

The pQE-9 vector is digested with *Bam* HI and *Hin* dIII and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the *E. coli* strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the *lacI* repressor and also confers kanamycin resistance (Kan<sup>R</sup>). Transformants are identified by their ability to grow on LB plates and colonies are selected which are resistant to both ampicillin and kanamycin. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sub>600</sub>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the *lacI* repressor, clearing the promoter/operator leading to increased gene expression.

Cells are grown for an additional 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000 X g). The cell pellet is solubilized in the chaotropic agent 6 M Guanidine-HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified IL-21 protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the IL-21 protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a

linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified IL-21 protein is stored at 4°C or frozen at -80°C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to an IL-21 polynucleotide, called pHE4a (ATCC Accession Number 209645, deposited February 25, 1998). This vector contains: (1) a neomycin phosphotransferase gene as a selection marker, (2) an *E. coli* origin of replication, (3) a T5 phage promoter sequence, (4) two lac operator sequences, (5) a Shine-Delgarno sequence, and (6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with *Nde* I and *Xba* I, *Bam* HI, *Xho* I, or *Asp* 718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers which encode restriction sites for *Nde* I (5' primer) and *Nde* I and *Xba* I, *Bam* HI, *Xho* I, or *Asp* 718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### Example 6: Purification of IL-21 Polypeptide from an Inclusion Body

The following alternative method can be used to purify IL-21 polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50



mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 x g centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 x g) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 µm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the IL-21 polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant IL-21 polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 µg of purified

protein is loaded. The purified IL-21 protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

5

**Example 7: Cloning and Expression of IL-21 in a Baculovirus Expression System**

10 In this example, the plasmid shuttle vector pA2 is used to insert IL-21 polynucleotide into a baculovirus to express IL-21. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as *Bam* HI, *Xba* I and *Asp* 718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the  
15 the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned IL-21 polynucleotide.

20 Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, by Luckow and colleagues  
25 (*Virology* 170:31-39 (1989)).

Specifically, the IL-21 cDNA sequence contained in the deposited clone, including the AUG initiation codon and any naturally associated leader sequence, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a  
30 second signal peptide. However, since the predicted naturally occurring signal peptides of IL-21 and IL-22 are not known, the vector can be modified (now designated pA2GP) to include a baculovirus leader sequence, using the standard methods described by Summers and coworkers ("A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin  
35 No. 1555 (1987)).

More specifically, the cDNA sequence encoding the full-length IL-21 protein in the deposited clone is amplified using PCR oligonucleotide primers corresponding to

the 5' and 3' sequences of the gene. The 5' primer has the sequence 5'-CGC CGC GGA TCC GCC ATC CGC ACG AGT GGA CAC GG-3' (SEQ ID NO:11) containing the *Bam* HI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells (shown in the primer sequence in italics; Kozak, M., *J. Mol. Biol.* 196:947-950 (1987)), a "C" residue to preserve the reading frame, and 16 nucleotides of the sequence of the complete IL-21 protein shown in Figure 1. The 3' primer has the sequence 5'-CGC GGT ACC CAC TGA ACG GGG CAG CAC GC-3' (SEQ ID NO:12) containing the *Asp* 718 restriction site followed by 20 nucleotides complementary to the 3' noncoding sequence in Figure 1.

10 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, CA). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, CA).

15 The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

20 Five µg of a plasmid containing the polynucleotide is co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner and colleagues (*Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987)). One µg of BaculoGold™ virus DNA and 5 µg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 µl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27°C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith (*supra*). An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced IL-21 protein.

#### **Example 8: Expression of IL-21 in Mammalian Cells**

IL-21 polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV-I, HIV-1 and the early promoter of the

cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, IL-21 polypeptide can be expressed in stable cell lines containing the IL-21 polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as *dhfr*, *gpt*, neomycin or hygromycin allows the identification and isolation of the transfected cells.

The transfected IL-21 gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest (see, e.g., Alt, F. W., *et al.*, *J. Biol. Chem.* 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., *Biochem. et Biophys. Acta*, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., *Biotechnology* 9:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS; Murphy, *et al.*, *Biochem. J.* 227:277-279 (1991); Bebbington, *et al.*, *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No. 209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, *et al.*, *Mol. Cell. Biol.*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart, *et al.*, *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites *Bam* HI, *Xba* I and *Asp* 718, facilitate the cloning of IL-21. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

IL-21 polynucleotide is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner, et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (for example, 50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100-200  $\mu$ M. Expression of IL-21 is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### Example 9: Protein Fusions of IL-21

IL-21 polypeptides are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of IL-21 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification (see Example 5; see also EP A 394,827; Traunecker, et al., *Nature*

331:84-86 (1988). Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time *in vivo*. Nuclear localization signals fused to IL-21 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the *Bam* HI cloning site. Note that the 3' *Bam* HI site should be destroyed. Next, the vector containing the human Fc portion is again restricted with *Bam* HI, linearizing the vector, and IL-21 polynucleotide, isolated by the PCR protocol described in Example 1, is ligated into this *Bam* HI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence (see, e.g., WO 96/34891).

#### Human IgG Fc region (SEQ ID NO:13):

GGGATCCGGAGCCCAATCTTCTGACAAAACACACATGCCACCGTGCCAGCACCTGAATTCGAGGGTGCACCGTCAGTC  
TTCCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGTGGTGAAGTGAAGCA  
CGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATTAATGCCAAGACAAAGCCCGGGAGGAGCAGT  
ACAACAGCAGCTACCGTGTGGTCAAGCTCCTCACCGTCTGCACCGAGTGGCTGAATGGCAAGGAGTACAAGTCAAGGTC  
TCCAACAAAGCCCTCCCAACCCCATCGAGAAAACCATCTCCAAGCCAAAGGCGAGCCCGAGAACCACAGGTGTACACCT  
GCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTACCGCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCG  
TGGAGTGGGAGAGCAATGGGAGCCGAGGAGCAACTACAAGACCACGCTCCCGTGGTGGACTCCGACGGCTCTTCTCTCTC  
TACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGATGAGGCTCTGCACAA  
CCACTACACCGAGAAGAGCTCTCCCTGTCTCCGGTAAATGAGTGGACGGCCGGACTCTAGAGGAT

#### Example 10: Production of an Antibody

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The antibodies of the present invention can be prepared by a variety of methods (see, Current Protocols, Chapter 2). For example, cells expressing IL-21 is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of IL-21 protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al.*, *Nature* 256:495 (1975); Kohler, *et al.*, *Eur. J. Immunol.* 6:511 (1976); Kohler, *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with IL-21 polypeptide or, more preferably, with a secreted IL-21 polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands and colleagues (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the IL-21 polypeptide.

Alternatively, additional antibodies capable of binding to IL-21 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the IL-21 protein-specific antibody can be blocked by IL-21. Such antibodies comprise anti-idiotypic antibodies to the IL-21 protein-specific



antibody and can be used to immunize an animal to induce formation of further IL-21 protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted IL-21 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For *in vivo* use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art (see, for review, Morrison, *Science* 229:1202 (1985); Oi, *et al.*, *BioTechniques* 4:214 (1986); Cabilly, *et al.*, U.S. Patent No. 4,816,567; Taniguchi, *et al.*, EP 171496; Morrison, *et al.*, EP 173494; Neuberger, *et al.*, WO 8601533; Robinson, *et al.*, WO 8702671; Boulianne, *et al.*, *Nature* 312:643 (1984); Neuberger, *et al.*, *Nature* 314:268 (1985)).

#### **Example 11: Production Of IL-21 Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing IL-21 polypeptide to be tested. This supernatant can then be used in the screening assays described subsequently in Examples 13-20.

First, dilute poly-D-lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (Phosphate Buffered Saline; w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50 µg/ml. Add 200 µl of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate the poly-D-lysine solution and rinse with 1 ml PBS. The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in 0.5 ml DMEM (Dulbecco's Modified Eagle Medium) supplemented with 4.5 G/L glucose, L-glutamine (12-604F Biowhittaker), 10% heat inactivated FBS (14-503F Biowhittaker), and 1x Penstrep (17-602E Biowhittaker). Let the cells grow overnight.

Following overnight incubation, mix together in a sterile solution basin: 300  $\mu$ l Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL) in each well of a 96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2  $\mu$ g of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50  $\mu$ l of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT for 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150  $\mu$ l Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by simultaneously performing the following tasks in a staggered fashion. Thus, hands-on time is cut in half, and the cells are not excessively incubated in PBS. First, person A aspirates the media from four 24-well plates of cells, and then person B rinses each well with 0.5-1ml PBS. Person A then aspirates the PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200  $\mu$ l of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Plates are then incubated at 37°C for 6 hours.

While cells are incubating, the appropriate media is prepared: either 1% BSA in DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; 4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; 0.070 mg/L of D-L-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L-Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na- $2\text{H}_2\text{O}$ ; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate;

11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mM glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 µl for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, again, preferably by two people, at the end of the incubation period. Person A aspirates the transfection media, while person B adds 1.5 ml of the appropriate media to each well. Incubate at 37°C for 45 or 72 hours, depending on the media used (1%BSA for 45 hours or CHO-5 for 72 hours).

On day four, using a 300 µl multichannel pipetter, aliquot 600 µl in one 1ml deep well plate and the remaining supernatant into a 2 ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the IL-21 polypeptide directly (e.g., as a secreted protein) or by IL-21 inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site ("GAS") elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in

many cell types though it has been found in T-helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

5       The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

10       The Jaks are activated by a wide range of receptors summarized in the Table below (adapted from review by Schidler and Darnell, *Ann. Rev. Biochem.* 64:621-51 (1995))). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; 15 and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (where "Xxx" represents any amino acid; SEQ ID NO:14)).

20       Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

25       Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (see Table below). Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

|                   | Ligand                           | tyk2 | JAKs |      | STATs | GAS(elements) or ISRE |
|-------------------|----------------------------------|------|------|------|-------|-----------------------|
|                   |                                  |      | Jak1 | Jak2 | Jak3  |                       |
| 5                 | <u>IFN family</u>                |      |      |      |       |                       |
|                   | IFN-a/B                          | +    | +    | -    | -     | 1,2,3                 |
|                   | IFN-g                            |      | +    | +    | -     | 1                     |
|                   | IL-10                            | +    | ?    | ?    | -     | 1,3                   |
| 10                | <u>gp130 family</u>              |      |      |      |       |                       |
|                   | IL-6 (Pleiotrohic)               | +    | +    | +    | ?     | 1,3                   |
|                   | IL-11(Pleiotrohic)               | ?    | +    | ?    | ?     | 1,3                   |
|                   | OnM(Pleiotrohic)                 | ?    | +    | +    | ?     | 1,3                   |
|                   | LIF(Pleiotrohic)                 | ?    | +    | +    | ?     | 1,3                   |
|                   | CNTF(Pleiotrohic)                | -/+  | +    | +    | ?     | 1,3                   |
| 15                | G-CSF(Pleiotrohic)               | ?    | +    | ?    | ?     | 1,3                   |
|                   | IL-12(Pleiotrohic)               | +    | -    | +    | +     | 1,3                   |
| 50099800950866009 | <u>g-C family</u>                |      |      |      |       |                       |
|                   | IL-2 (lymphocytes)               | -    | +    | -    | +     | 1,3,5                 |
|                   | IL-4 (lymph/myeloid)             | -    | +    | -    | +     | 6                     |
|                   | IL-7 (lymphocytes)               | -    | +    | -    | +     | 5                     |
|                   | IL-9 (lymphocytes)               | -    | +    | -    | +     | 5                     |
|                   | IL-13 (lymphocyte)               | -    | +    | ?    | ?     | 6                     |
|                   | IL-15                            | ?    | +    | ?    | +     | 5                     |
|                   | <u>gp140 family</u>              |      |      |      |       |                       |
| 30                | IL-3 (myeloid)                   | -    | -    | +    | -     | 5                     |
|                   | IL-5 (myeloid)                   | -    | -    | +    | -     | 5                     |
|                   | GM-CSF (myeloid)                 | -    | -    | +    | -     | 5                     |
| 35                | <u>Growth hormone family</u>     |      |      |      |       |                       |
|                   | GH                               | ?    | -    | +    | -     | 5                     |
|                   | PRL                              | ?    | +/-  | +    | -     | 1,3,5                 |
|                   | EPO                              | ?    | -    | +    | -     | 5                     |
| 40                | <u>Receptor Tyrosine Kinases</u> |      |      |      |       |                       |
|                   | EGF                              | ?    | +    | +    | -     | 1,3                   |
|                   | PDGF                             | ?    | +    | +    | -     | 1,3                   |
|                   | CSF-1                            | ?    | +    | +    | -     | 1,3                   |

To construct a synthetic GAS containing promoter element, which is used in the biological assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS-binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman, *et al.*, *Immunity* 1:457-468 (1994)), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18 bp of sequence complementary to the SV40 early promoter sequence and is flanked with an *Xho* I restriction site. The sequence of the 5' primer is: 5'-GCG CCT CGA GAT TTC CCC GAA ATC TAG ATT TCC CCG AAA TGA TTT CCC CGA AAT GAT TTC CCC GAA ATA TCT GCC ATC TCA ATT AG-3' (SEQ ID NO:15).

The downstream primer is complementary to the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA GGC-3' (SEQ ID NO:16).

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene). Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGT  
CAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCGCCCATCTCCGCCCATGGCTGA  
CTAATTTTATTTATTTATGTCAGAGGCGAGGCGGCTCGGCTCTGAGCTATCCAGAAGTAGTGAGGAGGCTTTTGGAGG  
CCTAGGCTTTTGCAAAAGCTT (SEQ ID NO:17).

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP". Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using *Hin* dIII and *Xho* I, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector

using *Sal* I and *Not* I, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NF- $\kappa$ B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HeLa (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

### Example 13: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity of IL-21 by determining whether IL-21 supernatant proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies; transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200  $\mu$ l of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies)

with 10 µg of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 µl of DMRIE-C and incubate at room temperature for 15-45 min.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37°C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with supernatants containing IL-21 polypeptides or IL-21 induced polypeptides as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 µl of cells into each well (therefore adding 100,000 cells per well).

After all the plates have been seeded, 50 µl of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 µl samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophane covers) and stored at -20°C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4°C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

#### Example 14: High-Throughput Screening Assay Identifying Myeloid Activity



The following protocol is used to assess myeloid activity of IL-21 by determining whether IL-21 proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12.

5 Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda, *et. al.*, *Cell Growth & Differentiation*, 5:259-265 (1994)) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

10

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8  $\mu$ g GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375  $\mu$ M  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675  $\mu$ M  $\text{CaCl}_2$ . Incubate at 37°C for 45 min.

15

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37°C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400  $\mu$ g/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400  $\mu$ g/ml G418 for couple of passages.

20

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200  $\mu$ l cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

25

Add 50  $\mu$ l of the supernatant prepared by the protocol described in Example 11. Incubate at 37°C for 48 to 72 hr. As a positive control, 100 U/ml interferon gamma can be used which is known to activate U937 cells. Over 30-fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

30

#### Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.

35

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by IL-21.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by IL-21 can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (nucleotides -633 to +1; Sakamoto, K., *et al.*, *Oncogene* 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers: (A) 5' Primer: 5'-GCG CTC GAG GGA TGA CAG CGA TAG AAC CCC GG-3' (SEQ ID NO:18) and (B) 3' Primer: 5'-GCG AAG CTT CGC GAC TCC CCG GAT CCG CCT C-3' (SEQ ID NO:19).

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes *Xho* I and *Hin* dIII, removing the GAS/SV40 stuffer fragment. Digest the EGR1 amplified product with the same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, 2 ml of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin on a precoated 10 cm tissue culture dish. A 1:4 split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 µg/ml G418. The G418-free medium is used for routine

growth but every one to two months, the cells should be re-grown in 300 µg/ml G418 for several passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS. Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5x10<sup>5</sup> cells/ml.

Add 200 µl of the cell suspension to each well of 96-well plate (equivalent to 1x10<sup>5</sup> cells/well). Add 50 µl supernatant produced by Example 11, 37° C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/µl of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-κB (Nuclear Factor κB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-α and lymphotoxin-β, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-κB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF-κB appears to shield cells from apoptosis), B- and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF-κB is retained in the cytoplasm with I-κB (Inhibitor κB). However, upon stimulation, I-κB is phosphorylated and degraded, causing NF-κB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF-κB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-κB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-κB would be useful in treating diseases. For example, inhibitors of NF-κB could be used to treat those diseases related to the acute or chronic activation of NF-κB, such as rheumatoid arthritis.

To construct a vector containing the NF- $\kappa$ B promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF- $\kappa$ B binding site (5'-GGG GAC TTT CCC-3'; SEQ ID NO:20), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an *Xho* I site: 5'-GCG GCC TCG AGG GGA CTT TCC CGG GGA CTT TCC GGG GAC TTT CCG GGA CTT TCC ATC CTG CCA TCT CAA TTA G-3' (SEQ ID NO:21).

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a *Hin* dIII site: 5'-GCG GCA AGC TTT TTG CAA AGC CTA GGC-3' (SEQ ID NO:22).

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with *Xho* I and *Hin* dIII and subcloned into BLSK2- (Stratagene). Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5'-CTCGAGGGGACTTTCCCGGGGACTTTCCCGGGGACTTTCCCGGACTTTCCATCTGCCATCTCAATTAGTCAGCAACCATAG  
TCCCGCCCTAACTCCGCCCCTCCCGCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCTAGGCTGACTAATTTT  
ATTTATCGAGAGCCGAGGCCGCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTGTGGAGGCTTAGGCTTTTG  
CAAAAAGCTT-3' (SEQ ID NO:23)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF- $\kappa$ B/SV40 fragment using *Xho* I and *Hin* dIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF- $\kappa$ B/SV40/SEAP cassette is removed from the above NF- $\kappa$ B/SEAP vector using restriction enzymes *Sal* I and *Not* I, and inserted into a vector containing neomycin resistance. Particularly, the NF- $\kappa$ B/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with *Sal* I and *Not* I.

Once NF- $\kappa$ B/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF- $\alpha$  (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

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### Example 17: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15  $\mu$ l of 2.5x dilution buffer into Optiplates containing 35  $\mu$ l of a supernatant. Seal the plates with a plastic sealer and incubate at 65°C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50  $\mu$ l Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50  $\mu$ l Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

**Table III: Reaction Buffer Formulation:**

| # of plates | Rxn buffer diluent (ml) | CSPD (ml) |
|-------------|-------------------------|-----------|
| 10          | 60                      | 3         |
| 11          | 65                      | 3.25      |
| 12          | 70                      | 3.5       |
| 13          | 75                      | 3.75      |
| 14          | 80                      | 4         |
| 15          | 85                      | 4.25      |
| 16          | 90                      | 4.5       |
| 17          | 95                      | 4.75      |
| 18          | 100                     | 5         |
| 19          | 105                     | 5.25      |
| 20          | 110                     | 5.5       |
| 21          | 115                     | 5.75      |
| 22          | 120                     | 6         |
| 23          | 125                     | 6.25      |
| 24          | 130                     | 6.5       |
| 25          | 135                     | 6.75      |
| 26          | 140                     | 7         |
| 27          | 145                     | 7.25      |
| 28          | 150                     | 7.5       |
| 29          | 155                     | 7.75      |
| 30          | 160                     | 8         |
| 31          | 165                     | 8.25      |
| 32          | 170                     | 8.5       |

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|    |     |       |
|----|-----|-------|
| 33 | 175 | 8.75  |
| 34 | 180 | 9     |
| 35 | 185 | 9.25  |
| 36 | 190 | 9.5   |
| 37 | 195 | 9.75  |
| 38 | 200 | 10    |
| 39 | 205 | 10.25 |
| 40 | 210 | 10.5  |
| 41 | 215 | 10.75 |
| 42 | 220 | 11    |
| 43 | 225 | 11.25 |
| 44 | 230 | 11.5  |
| 45 | 235 | 11.75 |
| 46 | 240 | 12    |
| 47 | 245 | 12.25 |
| 48 | 250 | 12.5  |
| 49 | 255 | 12.75 |
| 50 | 260 | 13    |

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

For adherent cells, seed the cells at 10,000-20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 µl of HBSS (Hank's Balanced Salt Solution) leaving 100 µl of buffer after the final wash.

A stock solution of 1 mg/ml fluo-3 is made in 10% pluronic acid DMSO. To load the cells with fluo-3, 50 µl of 12 µg/ml fluo-3 is added to each well. The plate is incubated at 37°C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 µl of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to  $2.5 \times 10^6$  cells/ml with HBSS in a 50-ml conical tube. Four  $\mu$ l of 1 mg/ml fluo-3 solution in 10% pluronic acid DMSO is added to each 1 ml of cell suspension. The tube is then placed in a 37°C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to  $1 \times 10^6$  cells/ml, and dispensed into a microplate, 100  $\mu$ l/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200  $\mu$ l, followed by an aspiration step to 100  $\mu$ l final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-3. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50  $\mu$ l. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either IL-21 or a molecule induced by IL-21, which has resulted in an increase in the intracellular  $Ca^{2+}$  concentration.

#### Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., *src*, *yes*, *lck*, *lyn*, *fyn*) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether IL-21 or a molecule induced by IL-21 is capable of

activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately  
 5 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or  
 10 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4°C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamar Blue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture  
 15 plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20  
 20 minutes, treatment with EGF (60ng/ml) or 50 µl of the supernatant produced in Example 11, the medium was removed and 100 µl of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating  
 25 shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 µm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is  
 30 removed and centrifuged for 15 minutes at 4°C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a  
 35 biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and



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PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10  $\mu$ l of 5  $\mu$ M Biotinylated Peptide, then 10  $\mu$ l ATP/Mg<sup>2+</sup> (5 mM ATP/50 mM MgCl<sub>2</sub>), then 10  $\mu$ l of 5x Assay Buffer (40 mM imidazole hydrochloride, pH 7.3, 40 mM  $\beta$ -glycerophosphate, 1 mM EGTA, 100 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5  $\mu$ l of Sodium Vanadate (1 mM), and then 5  $\mu$ l of water. Mix the components gently and preincubate the reaction mix at 30°C for 2 min. Initiate the reaction by adding 10  $\mu$ l of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10  $\mu$ l of 120 mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50  $\mu$ l aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37°C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide.

Wash the MTP module with 300  $\mu$ l/well of PBS four times. Next add 75  $\mu$ l of anti-phosphotyrosine antibody conjugated to horse radish peroxidase (anti-P-Tyr-POD (0.5  $\mu$ l/ml)) to each well and incubate at 37°C for one hour. Wash the well as above.

Next add 100  $\mu$ l of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 min (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1 ml of protein G (1  $\mu$ g/ml) for 2 hr at room temp (RT). The plates are

then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100 ng/well) against Erk-1 and Erk-2 (1 hr at RT; available from Santa Cruz Biotechnology). To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules. After 3-5 rinses with PBS, the plates are stored at 4°C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6 ng/well) or 50 µl of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10 ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1 µg/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by IL-21 or a molecule induced by IL-21.

#### Example 21: Method of Determining Alterations in the IL-21 Gene

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art (see, Sambrook, *et al.*, *supra*). The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:1. Suggested PCR conditions consist of 35 cycles at 95°C for 30 seconds; 60-120 seconds at 52-58°C; and 60-120 seconds at 70°C, using buffer solutions described by Sidransky and colleagues (*Science* 252:706 (1991)).

PCR products are then sequenced using primers labeled at the 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase (Epicentre Technologies). The intron-exon borders of selected exons of IL-21 are also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations in IL-21 are then cloned and sequenced to validate the results of the direct sequencing.

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PCR products of IL-21 are cloned into T-tailed vectors as described by Holton and Graham (*Nucl. Acids Res.* 19:1156 (1991)) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations in IL-21 not present in unaffected individuals.

5 Genomic rearrangements are also observed as a method of determining alterations in the IL-21 gene. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described by Johnson and coworkers (*Methods Cell Biol.* 35:73-99 (1991)). Hybridization with the labeled probe is carried out using a vast  
10 excess of human cot-1 DNA for specific hybridization to the IL-21 genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera  
15 (Photometrics, Tucson, AZ) and variable excitation wavelength filters (Johnson, C., *et al.*, *Genet. Anal. Tech. Appl.* 8:75 (1991)). Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC). Chromosome alterations of the genomic region of IL-21 (hybridized by the probe) are identified as insertions,  
20 deletions, and translocations. These IL-21 alterations are used as a diagnostic marker for an associated disease.

**Example 22: Method of Detecting Abnormal Levels of IL-21 in a**  
25 **Biological Sample**

IL-21 polypeptides can be detected in a biological sample, and if an increased or decreased level of IL-21 is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one  
30 skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect IL-21 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to IL-21, at a final concentration of 0.2 to 10 µg/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example  
35 10. The wells are blocked so that non-specific binding of IL-21 to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing IL-21. Preferably, serial dilutions of the sample should be used to validate

results. The plates are then washed three times with deionized or distilled water to remove unbound IL-21.

Next, 50  $\mu$ l of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature.

5 The plates are again washed three times with deionized or distilled water to remove unbound conjugate.

Add 75  $\mu$ l of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard  
10 curve, using serial dilutions of a control sample; and plot IL-21 polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the IL-21 in the sample using the standard curve.

#### 15 Example 23: Formulating a Polypeptide

The IL-21 composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the IL-21 polypeptide alone), the  
20 site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of IL-21 administered parenterally per dose will be in the range of about 1  $\mu$ g/kg/day to 10  
25 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, IL-21 is typically administered at a dose rate of about 1  $\mu$ g/kg/hour to about 50  $\mu$ g/kg/hour, either by 1-4 injections per day or by continuous subcutaneous  
30 infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing IL-21 are administered orally, rectally,  
35 parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid

filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

- 5 IL-21 is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U., *et al.*, *Biopolymers* 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer, R., *et al.*, *J. Biomed. Mater. Res.* 15:167-277 (1981); Langer, R. *Chem. Tech.* 12:98-105 (1982)), ethylene vinyl acetate (Langer, R., *et al.*) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped IL-21 polypeptides. Liposomes containing the IL-21 are prepared by methods known *per se* (DE 3,218,121; 15 Epstein, *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang, *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324). Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 20 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

- For parenteral administration, in one embodiment, IL-21 is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is 25 non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

- Generally, the formulations are prepared by contacting IL-21 uniformly and 30 intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are 35 also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at

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the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

IL-21 is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

IL-21 used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

IL-21 polypeptides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous IL-21 polypeptide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized IL-21 polypeptide using bacteriostatic Water-For-Injection (WFI).

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, IL-21 may be employed in conjunction with other therapeutic compounds.

**Example 24: Method of Treating Decreased Levels of IL-21**

5 The present invention relates to a method for treating an individual in need of a decreased level of IL-21 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of IL-21 antagonist. Preferred antagonists for use in the present invention are IL-21-specific antibodies.

10 Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of IL-21 in an individual can be treated by administering IL-21, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of IL-21 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of IL-21 to increase the activity level of IL-21 in such an individual.

15 For example, a patient with decreased levels of IL-21 polypeptide receives a daily dose 0.1-100  $\mu\text{g/kg}$  of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

#### 20 Example 25: Method of Treating Increased Levels of IL-21

The present invention also relates to a method for treating an individual in need of an increased level of IL-21 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of IL-21 or an agonist thereof.

25 Antisense technology is used to inhibit production of IL-21. This technology is one example of a method of decreasing levels of IL-21 polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

30 For example, a patient diagnosed with abnormally increased levels of IL-21 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### 35 Example 26: Method of Treatment Using Gene Therapy

One method of gene therapy transplants fibroblasts, which are capable of expressing IL-21 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37°C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T., *et al.*, *DNA* 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with *Eco* RI and *Hin* dIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding IL-21 can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1. Preferably, the 5' primer contains an *Eco* RI site and the 3' primer includes a *Hin* dIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified *Eco* RI and *Hin* dIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains properly inserted IL-21.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the IL-21 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the IL-21 gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media



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from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether IL-21 protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference.

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***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209666;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (g) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666;
- (h) a polynucleotide encoding a polypeptide of SEQ ID NO:2 or the cDNA sequence included in ATCC Deposit No: 209666 having biological activity;
- (i) a polynucleotide which is a variant of SEQ ID NO:1;
- (j) a polynucleotide which is an allelic variant of SEQ ID NO:1;
- (k) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:2;
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; and
- (m) a polynucleotide which is the complement of any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k) or (m).

2. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:28;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:28;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:28;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:28;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:28;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:28;
- (g) a polynucleotide encoding conserved polypeptide domain V of SEQ ID NO:28;
- (h) a polynucleotide encoding conserved polypeptide domain VI of SEQ ID NO:28;
- (i) a polynucleotide encoding conserved polypeptide domain VII of SEQ ID NO:28;
- (j) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:28;
- (k) a polynucleotide encoding a polypeptide of SEQ ID NO:28 having biological activity;
- (l) a polynucleotide which is a variant of SEQ ID NO:28;
- (m) a polynucleotide which is an allelic variant of SEQ ID NO:28;
- (n) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:28;
- (o) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m) or (n), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; and
- (p) a polynucleotide which is the complement of any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k), (l), (m), (n) or (o).

3. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polynucleotide fragment of SEQ ID NO:3 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: 209665;
- (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (c) a polynucleotide encoding conserved polypeptide domain I of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (d) a polynucleotide encoding conserved polypeptide domain II of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (e) a polynucleotide encoding conserved polypeptide domain III of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (f) a polynucleotide encoding conserved polypeptide domain IV of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (g) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665;
- (h) a polynucleotide encoding a polypeptide of SEQ ID NO:4 or the cDNA sequence included in ATCC Deposit No: 209665 having biological activity;
- (i) a polynucleotide which is a variant of SEQ ID NO:3;
- (j) a polynucleotide which is an allelic variant of SEQ ID NO:3;
- (k) a polynucleotide which encodes a species homologue of the polypeptide whose amino acid sequence is shown in SEQ ID NO:4;
- (l) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j) or (k), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; and
- (m) a polynucleotide which is the complement of any one of the polynucleotides specified in (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), (k) or (l).

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

5. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

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6. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a mature form or a secreted protein.

7. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:2 or the coding sequence included in ATCC Deposit No: 209666.

8. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:28.

9. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:4 or the coding sequence included in ATCC Deposit No: 209665.

10. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1 or the cDNA sequence included in ATCC Deposit No: 209666.

11. The isolated nucleic acid molecule of claim 2, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:28.

12. The isolated nucleic acid molecule of claim 3, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:3 or the cDNA sequence included in ATCC Deposit No: 209665.

13. The isolated nucleic acid molecule of claims 4, 5 or 6, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

14. The isolated nucleic acid molecule of claims 7, 8 or 9, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

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15. A recombinant vector comprising the isolated nucleic acid molecule of claims 1, 2 or 3.

16. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

17. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 2.

18. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 3.

19. A recombinant host cell produced by the method of claim 16.

20. A recombinant host cell produced by the method of claim 17.

21. A recombinant host cell produced by the method of claim 18.

22. The recombinant host cell of claim 19 comprising vector sequences.

23. The recombinant host cell of claim 20 comprising vector sequences.

24. The recombinant host cell of claim 21 comprising vector sequences.

25. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(b) a polypeptide fragment of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666 having biological activity;

(c) a polypeptide domain of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(d) a polypeptide epitope of SEQ ID NO:2 or the encoded sequence included in ATCC Deposit No: 209666;

(e) a mature form of a secreted protein;

(f) a full length secreted protein;

(g) a variant of SEQ ID NO:2;

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- (h) an allelic variant of SEQ ID NO:2; and
- (i) a species homologue of the SEQ ID NO:2.

26. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:29;
- (b) a polypeptide fragment of SEQ ID NO:29 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:29;
- (d) a polypeptide epitope of SEQ ID NO:29;
- (e) a mature form of a secreted protein of SEQ ID NO:29;
- (f) a full length secreted protein of SEQ ID NO:29;
- (g) a variant of SEQ ID NO:29;
- (h) an allelic variant of SEQ ID NO:29; and
- (i) a species homologue of the SEQ ID NO:29.

27. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (b) a polypeptide fragment of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665 having biological activity;
- (c) a polypeptide domain of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (d) a polypeptide epitope of SEQ ID NO:4 or the encoded sequence included in ATCC Deposit No: 209665;
- (e) a mature form of a secreted protein;
- (f) a full length secreted protein;
- (g) a variant of SEQ ID NO:4;
- (h) an allelic variant of SEQ ID NO:4; and
- (i) a species homologue of the SEQ ID NO:4.

28. The isolated polypeptide of claims 26, 26 or 27, wherein the mature form or the full length secreted protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

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29. An isolated antibody that binds specifically to the isolated polypeptide of claims 25, 26 or 27.
30. A recombinant host cell that expresses the isolated polypeptide of claim 25.
31. A recombinant host cell that expresses the isolated polypeptide of claim 26.
32. A recombinant host cell that expresses the isolated polypeptide of claim 27.
33. A method of making an isolated polypeptide comprising:
  - (a) culturing the recombinant host cell of claim 30 under conditions such that said polypeptide is expressed; and
  - (b) recovering said polypeptide.
34. A method of making an isolated polypeptide comprising:
  - (a) culturing the recombinant host cell of claim 31 under conditions such that said polypeptide is expressed; and
  - (b) recovering said polypeptide.
35. A method of making an isolated polypeptide comprising:
  - (a) culturing the recombinant host cell of claim 32 under conditions such that said polypeptide is expressed; and
  - (b) recovering said polypeptide.
36. The polypeptide produced by claims 33, 34 or 35.
37. A method for preventing, treating, or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of the polypeptide of claims 25, 26 or 27 or of the polynucleotide of claims 1, 2 or 3.
38. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:



(a) determining the presence or absence of a mutation in the polynucleotide of claims 1, 2 or 3;

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

39. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject related to expression or activity of a secreted protein comprising:

(a) determining the presence or amount of expression of the polypeptide of claims 25, 26 or 27 in a biological sample;

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

40. A method for identifying binding partner to the polypeptide of claims 25, 26 or 27 comprising:

(a) contacting the polypeptide of claims 25, 26 or 27 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

41. The gene corresponding to the cDNA sequence of SEQ ID NO:1.

42. The gene corresponding to the cDNA sequence of SEQ ID NO:28.

43. The gene corresponding to the cDNA sequence of SEQ ID NO:3.

44. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:1 in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

45. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:28 in a cell;

- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

46. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:3 in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

47. The product produced by the method of claim 44.

48. The product produced by the method of claim 45.

49. The product produced by the method of claim 46.

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### Abstract

5. The present invention relates to novel human proteins designated Interleukin-21 (IL-21) and Interleukin-22 (IL-22), and isolated polynucleotides encoding these proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human proteins.

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ccacgcctcc cgtgctggac tccgacggct ccttcttctt ctacagcaag ctcaccgtgg 600  
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gcccctaact ccgcccagtt ccgcccattc tccgcccatt ggctgactaa ttttttttat 180

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cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga 180
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aaggcgtgga ctcaccgctg ggtgcttgc aaanaaggata gggacgcata tgctttttaa 180  
agcaatctaa aaataataat aagtatagcg actatatacc tactttttaa atcaactgtt 240  
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ggnttcggtc ggcgactctg aagagagtnc accgagcaaa ccaagtgccg gagcaacagc 240  
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tttttagtta caagcaagca nntggnttga agtngntggg gaaaggancc gnagggattc 360  
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 Leu Phe Leu Thr Trp Leu His Thr Cys Leu Ala His His Asp Pro Ser  
 10 15 20  
 ctc agg ggg cac ccc cac agt cac ggt acc cca cac tgc tac tgc gct 150  
 Leu Arg Gly His Pro His Ser His Gly Thr Pro His Cys Tyr Ser Ala  
 25 30 35  
 gag gaa ctg ccc ctc ggc cag gcc ccc cca cac ctg ctg gct cga ggt 198  
 Glu Glu Leu Pro Leu Gly Gln Ala Pro Pro His Leu Leu Ala Arg Gly  
 40 45 50 55  
 gcc aag tgg ggg cag gct ttg cct gta gcc ctg gtg tcc agc ctg gag 246  
 Ala Lys Trp Gly Gln Ala Leu Pro Val Ala Leu Val Ser Ser Leu Glu  
 60 65 70  
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 Ala Ala Ser His Arg Gly Arg His Glu Arg Pro Ser Ala Thr Thr Gln  
 75 80 85  
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 Cys Pro Val Leu Arg Pro Glu Glu Val Leu Glu Ala Asp Thr His Gln  
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 Arg Ser Ile Ser Pro Trp Arg Tyr Arg Val Asp Thr Asp Glu Asp Arg  
 105 110 115  
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 Tyr Pro Gln Lys Leu Ala Phe Ala Glu Cys Leu Cys Arg Gly Cys Ile  
 120 125 130 135  
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360160.50856099

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140      145      150
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Leu Gln Ser Leu Leu Val Leu Arg Arg Arg Pro Cys Ser Arg Asp Gly
155      160      165

tcg ggg ctc ccc aca cct ggg gcc ttt gcc ttc cac acc gag ttc atc 582
Ser Gly Leu Pro Thr Pro Gly Ala Phe Ala Phe His Thr Glu Phe Ile
170      175      180

cac gtc ccc gtc ggc tgc acc tgc gtg ctg ccc cgt tca gtg 624
His Val Pro Val Gly Cys Thr Cys Val Leu Pro Arg Ser Val
185      190      195

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20      25      30
Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly Gln Ala Pro
35      40      45
Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln Ala Leu Pro Val
50      55      60
Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His Arg Gly Arg His Glu
65      70      75      80
Arg Pro Ser Ala Thr Thr Gln Cys Pro Val Leu Arg Pro Glu Glu Val
85      90      95
Leu Glu Ala Asp Thr His Gln Arg Ser Ile Ser Pro Trp Arg Tyr Arg

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50099805-091098

100 105 110  
Val Asp Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu Ala Phe Ala Glu  
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130 135 140  
Ala Leu Asn Ser Val Arg Leu Leu Gln Ser Leu Leu Val Leu Arg Arg  
145 150 155 160  
Arg Pro Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe  
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agagcctgac tggtgctgcy ccgccggccc tgactaccgc cnacggacta cgggggctac 180  
cccacacctg gggncctttg acctccaca ccgnagttac atgccacgta ccccgttcgg 240  
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ccctnagtac tggnacacgt gtgatacccc ag 332

860160-5886608

Figure 1  
Interleukin-21

1 GGCACGAGTGGACACGGATGAGGACCGCTATCCACAGAAGCTGGCCTTCGCCGAGTGCCT 60  
1 A R V D T D E D R Y P Q K L A F A E C L 20  
Domain I Domain II

61 GTGCAGAGGCTGTATCGATGCAAGGACGGGCGGAGACAGCTGGGCTCAACTCCGTGCG 120  
21 C R G C I D A R T G R E T A A L N S V R 40  
Domain II

121 GCTGCTCCAGACCTGCTGGTGTGCGCGCGCGCCCTGCTCCCGGACGGCTGGGGCT 180  
41 L L Q S L L V L R R R P C S R D G S G L 60  
Domain III

181 CCCACACCTGGGSCCTTGCCTTCCACACCGAGTTCATCCACGTCCCGCTGGGTGCAC 240  
61 P T P G A F A F H T E F I H V P V G C T 80  
Domain IV

241 CTGGTGCTGCCCCGTTCACTGTGACCGCCCAAGCCCGTGGGGCCCTTAGACTGGACACGT 300  
81 C V L P R S V 87  
Domain IV

301 GTGCTCCCCAGAGGGCACCCCTATTATGTGTATTATTGTATTATTATATGCTCCCC 360

361 AACACTACCTTGGGGTCTGGGCATTCCCCGTGTCTGGAGGACAGCCCCCACTGTCTC 420

421 CTCATCTCCAGCCTCAGTAGTTGGGGTGAAGGAGCTCAGCACCTCTTCCAGCCCTTAA 480

481 AGCTGCAGAAAAGGTGTACACGGCTGCCTGTACCTTGGYTCCCTGTCTGCTCCCGGT 540

541 TCCCTTACCTTATCACTGGGCTCAGGCCCCCGCAGGCTGCCTCTTCCCAACCTCCTTGA 600

601 AGTACCCCTGTCTTCTAAACAATTATTTAAGTGTACGTGTATTATTAACTGATGAACAC 660

661 AA 705

869160-50866009



Figure 2A  
Interleukin-22

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1  GGAATTGCGCACGAGCTCGTGCCTGCTCAGTGCCTTCCACCACAGCTGCAGCTGGGGC 60
1  N S A R A R A V L S A F H H T L Q L G P 20

#

61  CGCGTGAGCAGGCGCGCAACGCGAGCTGCCCGGAGGGGGCAGGCGCGACCGCGCT 120
21  R E Q A R N A S C P A G G R P A D' R R P 40

121  TCCGGCCGCCACCAACCTGCGCAGCGTGTGCGCCCTGGGGCTACAGAATCTCTACGACC 180
41  R P P T N L R S V S P W A Y R I S Y D P 60
                                     Domain I

181  CGCGAGGTACCCAGGTACCTGCGCTGAAGCCTACTGCTGTGCGGGGGCTGCTGACCG 240
61  A R Y P R Y L P E A Y C L C R G C L T G 80
      Domain I                               Domain II

241  GGCTGTTCGGCGAGGAGGAGCTGCGCTTCCGAGCGCCCTGTCTACATGCCCAACCGTCG 300
81  L F G E E D V R F R S A P V Y M P T V V 100
                                     Domain III

301  TCCTGCGCGCACCCCGCTGCGCGCGCGCGCTTCCGTCTACACCGAGGCTACGTCA 360
101 L R R T P A C A G G R S V Y T E A Y V T 120
      Domain III

#

361  CCATCCCGTGGGCTGCACCTGCGTCCCGAGCGGAGGAGGAGCGAGACATCAACT 420
121 I P V G C T C V P E P E K D A D S I N S 140
      Domain IV

421  CCAGCATCGACAAACAGGGGCGCAAGCTCCTGCTGGGCCCCAAGCAGCGCGCGCTGGCC 480
141 S I D K Q G A K L L L G P N D A P A G P 160

481  CCTGAGGCGGTCTCTGCCCGGGAGGTCTCCCGGCGCGCATCCCGAGGCGCCCAAGCTG 540

541  GAGCCGCTTGAGGGCTCGGTGCGGACCTCTGAAGAGAGTGCAACGAGCAAACCAAGTG 600

601  CCGGAGCACCAGCGCGCGCTTCCATGGAGACTCGTAAGCAGCTTCATCTGACACGGGCA 660

661  TCCTGGCTTGCTTTTAGCTACAAGCAAGCAGCGTGGCTGGAAGCTGATGGGAAACGACC 720

721  CGGCACGGGCATCCTGTGTGCGGCGCGCATGGAGGGTTTGAAAAAGTTACCGAGGCTCC 780

781  CTGAGGAGCCTCTCAGATCGGCTGCTGCGGGTGCGAGGCGTGACTCACCGCTGGGTGCTT 840

841  GCCAAAGAGATAGGACGCATATGCTTTTAAAGCAATCTAAAAATAATAAAGTATAG 900

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860160-5086609

Figure 2B  
Interleukin-22

901 CGACTATATACCTACTTTTAAATCAACTGTTTGAATAGAGGCAGAGCTATTTATATT 960  
961 ATCAAATGAGAGCTACTCTGTACATTTCTTAACATATAACATCGTTTTTACTTCTTC 1020  
1021 TGSTAGAATTTTTTAAAGCATAATTGAATCCTTGGATAAAATTTGTAGCTGGTACACTC 1080  
1081 TGCCCTGGGTCTCTGAATTCAGCCTGTCAACGATGGCTGACTGTGAAATGGACACGTCT 1140  
1141 CATCTGACCCACTCTCTCCCTCCACTGAAGGTCTTCACGGGCTCCAGGTGGACCAAAGGG 1200  
1201 ATGCACAGGCGGCTCGCATGCCCCAGGGCCAGCTAAGAGTTCCAAAGATCTCAGATTTGG 1260  
1261 TTTTAGTCATGAATACATAAACAGTCTCAAACCTGCACAAATTTTTCCCCCTTTTGAAAG 1320  
1321 CCACTGGGGCCAATTTGTGGTTAAGAGGTGGTGAGATAAGAAGTGAACGTGACATCTTT 1380  
1381 GCCAGTTGTGAGAAGAATCCAAGCAGGTATTGGCTTAGTTGTAAAGGGCTTTAGGATCAGG 1440  
1441 CTGAATATGAGGACAAAGTGGGCCACGTTAGCATCTGCAGAGATCAATCTGGAGGCTTCT 1500  
1501 GTTCTGCAATCTGCCACGAGAGCTAGGTCCTTGATCTTTCTTTAGATTGAAAGTCTGT 1560  
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1621 GCTGAAAAAAAAAAAAAAAAAAAA 1642

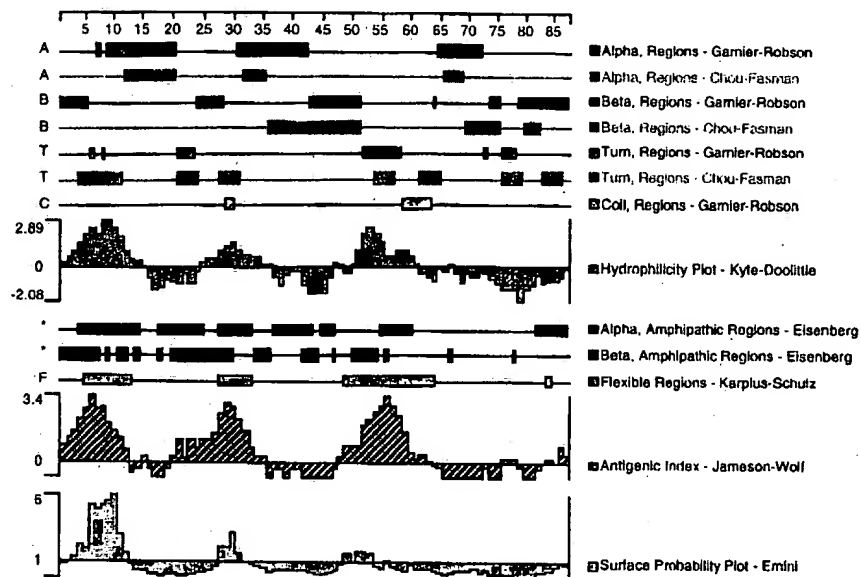
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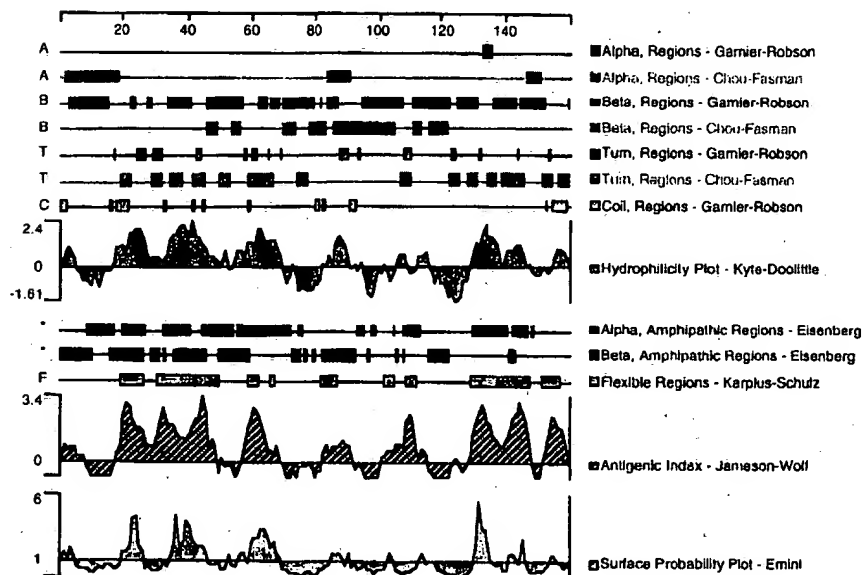
Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Figure 4  
Interleukin-21 Polypeptide Analysis



8601605096098

Figure 5  
Interleukin-22 Polypeptide Analysis



860160-50866009

Figure 6A  
Interleukin-21

1 GCTCCAAGCCAGCCTGCCCGCTGCCGCCACCATGACGCTCCCTCCCGGCTCCTGTTT 60  
1 M T L L P G L L F 9

61 CTGACCTGGCTGCACACATGCCCTGGCCACCATGACCCCTCCCTCAGGGGGCACCCCCAC 120  
10 L T W L H T C L A H H D P S L R G H P H 29

121 AGTCACGGTACCCACACTGCTACTCGGCTGAGGAAGTGCCTCCCTCAGGCCAGGCCCCCA 180  
30 S H G T P H C Y S A E E L P L G Q A P P 49  
Domain V

181 CACCTGCTGGCTCGAGGTGCCAAGTGGGGCAGGCTTTGCCTGTAGCCCTGGTGTCACG 240  
50 H L L A R G A K W G Q A L P V A L V S S 69  
Domain VI

241 CTGGAGGCAGCAAGCCACAGGGGAGGACGAGAGGCCCTCAGCTACGACCCAGTGCCCG 300  
70 L E A A S H R G R H E R P S A T T Q C P 89

301 GTGCTGCCGCGGAGGAGGTGTGGAGGCAGACACCCACGCGCTCCATCTCACCCCTGS 360  
90 V L R P E E V L E A D T H Q R S I S P W 109  
Domain VII

361 AGATACCGGTGGACACGGATGAGGACCGCTATCCACAGAGCTGGCCTTCGCCGAGTGC 420  
110 R Y R V D T D E D R Y P Q K L A F A E C 129  
Domain I Domain II

421 CTGTGCAGAGCCTGTATCGATGCACGGACGGCCGCGAGACAGCTGCGCTCACTCCGTG 480  
130 L C R G C I D A R T G R E T A A L N S V 149  
Domain II

481 CGGCTGCTCCAGAGCCTGCTGGTGTGCGCGCGCGCCCTGCTCCCGGACGGCTCGGG 540  
150 R L L Q S L L V L R R R P C S R D G S G 169  
Domain III

541 CTCCCCACACCTGGGGCCTTTGCCTTCCACACCGAGTTCATCCACGTCCTCCGCTGGCTGC 600  
170 L P T P G A F A F H T E F I H V P V G C 189  
Domain IV

601 ACCTGCGTGTGCCCCGTTTCACTGTGACCGCAAGGCCGCTGGGGCCCTTAGACTGGACAC 660  
190 T C V L P R S V 197  
Domain IV

661 GTGTGCTCCCAGAGGGCACCCCTATTATGTGTATTATTGTATTATATATGCTCCC 720

721 CCAACACTACCTTGGGGTCTGGGCATTCCCGTGTCTGGAGGACAGCCCCCACTGTT 780

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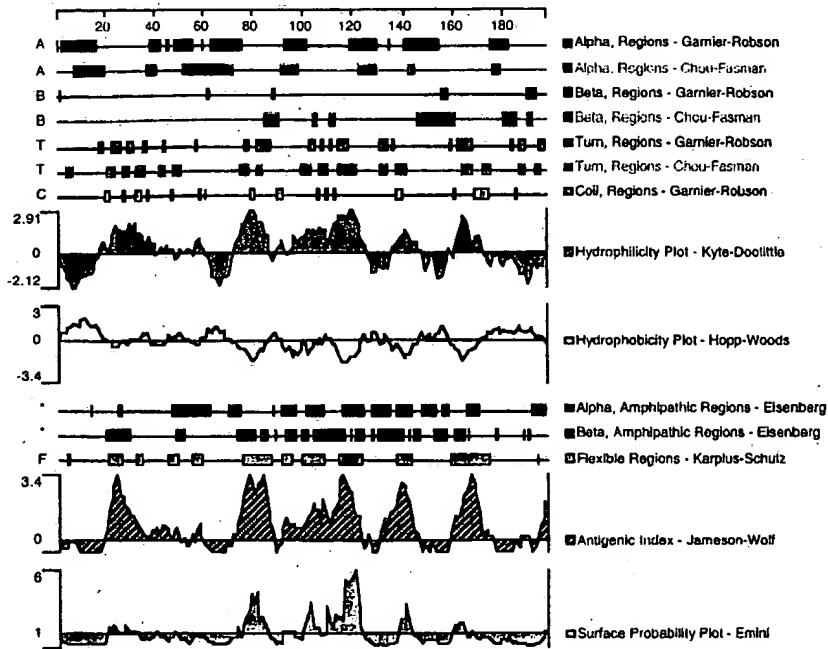
Figure 6B  
Interleukin-21

781 TCCTCATCTCCAGCCTCAGTAGTTGGGGTGAAGGAGCTCAGCACCTCTTCCAGCCCTT 840  
841 AAAGCTGCAGAAAAGGTGTACACGGCTGCCTGTACCTTGGYTCCCTGTCTGCTCCCGG 900  
901 CTTCCTTACCCTATCACTGGCCTCAGGCCCGCAGGCTGCCTCTTCCCAACCTCCTTG 960  
961 GAAGTACCCCTGTTCCTTAAACAATTATTAAAGTGTACGTATTATTAAACTGATGAAC 1020  
1021 AA 1067

860169.5886009



Figure 7  
Interleukin-21 Polypeptide Analysis



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PTO/SS.62 (37-CF)

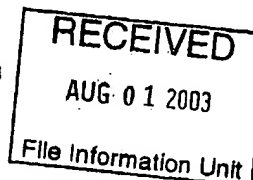
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US 20030003545A1

(19) **United States**(12) **Patent Application Publication** (10) Pub. No.: **US 2003/0003545 A1****EBNER et al.**(43) Pub. Date: **Jan. 2, 2003**(54) **INTERLEUKINS-21 AND 22**

Publication Classification

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**C07H 21/04; C12N 15/09;**  
**C12P 21/02; C12P 21/04;**  
**A61K 45/00; A61K 39/395;**  
**C12N 15/00; C12N 15/70;**  
**C12N 15/74; C12N 5/00;**  
**C12N 5/02; C07K 14/00;**  
**C07K 1/00; C07K 17/00**(52) U.S. Cl. .... **435/69.5; 435/6; 435/69.2;**  
**435/325; 435/320.1; 536/23.5;**  
**424/143.1; 424/85.2; 435/69.52;**  
**424/130.1; 530/351**

(\*) Notice: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

(21) Appl. No.: **09/320,713**(57) **ABSTRACT**(22) Filed: **May 27, 1999****Related U.S. Application Data**

(60) Provisional application No. 60/087,340, filed on May 29, 1998. Provisional application No. 60/099,805, filed on Sep. 10, 1998. Provisional application No. 60/131,965, filed on Apr. 30, 1999.

The present invention relates to novel human proteins designated Interleukin-21 (IL-21) and Interleukin-22 (IL-22), and isolated polynucleotides encoding these proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human proteins.



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